HORMONES, BRAIN FUNCTION, AND BEHAVIOR

# HORMONES, BRAIN FUNCTION, AND BEHAVIOR

*Proceedings of a Conference on Neuroendocrinology Held at Arden House, Harriman, New York, 1956*

Edited by

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# Foreword

This book constitutes the report of a conference on neuroendocrinology held in May, 1956, at Columbia University's Arden House on the Hudson. Twelve papers were presented on aspects of neuroendocrinology during the two-day meetings Each paper was followed by informal discussion

Recent years have shown a renaissance of interest in neuropharmacology resulting in large measure from the discovery of new drugs, especially lysergic acid diethylamide, that produce experimental psychotic episodes in man. The discovery of the value of tranquilizing drugs in relation to mental disorders has also added impetus to studies of biochemical determinants of conduct. The hormones are endogenously produced drugs regulating the internal environment of the cells and *modifying many aspects of behavior.*

Communication between cells and between organs may be classified roughly into two major categories: (1) communication by nerve impulses which are efficient discrete messages in the form of waves of electrochemical change coursing over specific fibers from sensory receptors to cells of the central nervous system, from cell to cell within this integrating system, and thence out to effector organs, (2) intercellular and interorgan communication either by short-distance diffusion of metabolites from cell to cell or by transportation by the blood stream to remote organs of metabolites such as $CO_2$ as well as the special metabolites of endocrine glands.

Such metabolic messengers which include the hormones have been referred to by Norbert Wiener as "to whom it may concern" messages Formed in glands of internal secretion and released into the blood stream, the hormones may be carried to all the tissues of the body before becoming inactive metabolites Relatively few of the molecules so broadcast reach target organs where they react with appropriate chemical receptors and so act as regulating messengers affecting tissue and organ function The papers and discussions of this symposium are aimed at elucidating selected aspects of hormone actions in relation to brain function and behavior.

The symposium was made possible by generous aid from the G. D. Searle Company of Chicago. We are especially indebted to Dr. I C.

Winter, Director of Clinical Research of the Searle Company, and his staff for assistance in organizing the conference

As chairman of the conference, I wish to express my sincere appreciation not only to the contributors of the papers but also to those participants whose lively and spontaneous discussion did so much to make the meetings profitable to all who attended.

HUDSON HOAGLAND

*Shrewsbury, Massachusetts*
*November 1956*

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# EFFECTS OF STEROID HORMONES ON THE NERVOUS SYSTEM

# Steroid Hormones in Relation to Neuropsychiatric Disorders

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## I INTRODUCTION

The more one pries into the bewildering complex of interactions between mind and bodily functions the less one feels able to name precise mechanisms responsible for any one disturbance. This has been said in many ways, many times, but perhaps not more aptly than in the perplexed words of Dr William Buchan of Edinburgh in his treatise on disease, in 1779 (12) In this he says, "The passions have great influence both in the cause and cure of diseases How mind acts upon matter will, in all probability, ever remain a secret It is sufficient for us to know that there is established a reciprocal influence betwixt the mental and corporeal parts, and that whatever disorders the one, likewise affects the other."

The history of man is, however, illuminated with repeated examples of refusal to accept obscurity as the answer to his endeavors to fathom and control his environment and himself So we see the gradual accretion of knowledge bringing more and more of the universe within his ken The development of endocrinology, which arrived rather late on the biological scene, is now contributing to an understanding of man himself (18) As it develops, it is becoming increasingly apparent that the endocrines are almost inextricably associated in function with that other major homeostatic regulator, the autonomic nervous system, in both central and peripheral activities (19) But as Mirsky (61) has said, "The paucity of information that is available concerning the specific role of humoral agents in the regulation of behaviour is due, primarily, to the fact that it is impossible to distinguish where one regulatory mechanism stops and another starts." Consequently, in this paper attention will be given to certain neurogenic functions which might, at first sight, be considered irrelevant to steroids and mental disorder. Old, as well as newer, data will also be reviewed, in the hope that they may suggest fresh points of departure for investigation in this field which promises so much but presently seems to yield so little of a definitive nature

## II. NATURAL ALTERATIONS IN CEREBRAL FUNCTION ELICITED BY STEROIDS

*Estrogens* contribute to certain regulatory functions of the hypothalamus (20). For example, the release of lutinizing hormone (LH) by the adenohypophysis can be advanced 24 hours in the 5-day cyclic rat by giving progesterone on the third day of diestrus, this being signified by ovulation. In the pregnant rat, *precocious ovulation*, which reflects LH release, can be precipitated by estrogen given on the fourth day of pregnancy, ovulation occurring 36 hours later. These experiments of Markee *et al.* (60) show that these steroids facilitate LH release by lowering the threshold of a sex center which appears to lead to the liberation of an adrenergic neurohumor which suitable blocking agents inhibit.

The above experiments represent steroid influence on discrete hypothalamic mechanisms. Relatively precise information also exists in support of the view that steroid hormones influence the participation of the hypothalamus in the control of more complex acts associated with mating For example, progesterone, in a dose well below the subcutaneously effective level, if placed in the lateral ventricle leads to estrous behavior in a castrate hamster (55). Further, it has been shown that lesions in the posterior hypothalamus of the guinea pig preclude mating responses elicited by estrogens, presumably because the integrating area for estral behavior has been disturbed (28).

*Androgens* have not been so definitively studied, but in the young dog the micturition pattern has been observed in relationship to sex and testicular function. Berg (7) observed that the adult male act of leg elevation while urinating did not appear till 19 weeks, but could be prevented by castration or advanced to 8 weeks by androgen injections. This is an instance of activation of an innate pattern.

*Courtship and mating* in mammals has associated much complex integrated behavior that has been described often, but in particular connection with hormones in the monograph by Beach (5). In these acts, steroids play an essential role in developing the pattern which, once established, can be very resistant to extinction in certain species, and especially in the male, despite gonadectomy In man, the strength of the sexual drive of the adult is reinforced by gonadal influences, but the direction is not. It is not possible to correct homosexual behavior by hormones, for instance The detailed morphologic and psychoanalytic studies of Benedek and Rubinstein (6) have demonstrated the delicate dependence of sex drives and attitudes, at conscious and unconscious

levels, on ovarian secretion and their inter-dependence on psychological factors. In the human, hormones definitely play a less defined role in sexuality. Castration in adults does not always lead to a loss of interest in sex (87), though it leads to an altered endopsychic concept of the self (88). Psychotherapy alone may enhance sexual expression in castrates (27), though this does not deny the validity of a similar result achieved by testosterone.

*Aggression* is of no less importance in man and animals than sexuality as a major drive, but, if anything, it is less well understood. Without exploring the well-documented interrelationships between the two, attention will now be paid to some observations which bear more particularly on generalized aggression, which has been reviewed in some detail by Beach (5). One experiment which seems most pertinent for mention here is that conducted by Clark and Birch (16) on dominance in adult male chimpanzees Using a food-reward situation, it was found that methyl testosterone increased dominance in the eunuchoid male, while estrogen led to a subordinate status

Estrogens have been used in humans for the suppression of assaultiveness associated with heterosexual offenses or bothersome homosexual trends, stilbestrol being used as a rule. It is apparently capable of controlling the aggression and, unlike castration, appears to abolish libido during the period of administration (30, 82, 83, 86). While testosterone may increase aggression in castrates, it has not been extensively exploited for this purpose in intact males In the last few years, several reports have appeared indicating the usefulness of epi-dehydro-isoandrosterone (diandrone), an adrenal cortical steroid. This substance allegedly comprises 8–15% of the 17-ketosteroids Studies in schizophrenic patients by Reiss *et al.* (73), led to its use. Trials of diandrone on a few adult schizophrenics and schizoid psychopaths showing a low beta excretion, by Strauss (86), gave a few favorable responses. More decisive results were obtained by others (83, 96) in immature adolescents. Sands (82) gives as indications the presence of timidity, lack of confidence, apathy, in young, inadequate psychopathic types. Aggression is promoted, but sex effects are not apparent. More definitive studies will have to be made in order to establish the validity of these claims, which have both theoretical and practical importance. The substance would seem to supplement a lack which has wide psychological repercussions

*Genetic factors* would seem to be operative in the control of what may liberally be termed aggressive behavior, in lower forms at least. For example, the behavior of the laboratory Norway rat is one of compliance,

within reason, but in the short space of a hundred years since it was first utilized for experimental purposes, it has changed mightily. Its wild cousin, as collected in Baltimore and studied by Richter (76), is a different animal. Untamable, vicious, and suspicious, it would appear to bear these characteristics in its germ plasm, for the young, foster-mothered by tame laboratory types, grow up true to their wild forebears. Endocrinological characteristics of the wild strain include small pituitaries and enormous adrenals. While not endeavoring to suggest that the glandular morphology affects the behavior, the association is worth noting. The genetics has been neglected, and only now receives revived attention. It is of current interest that a recent paper provides evidence that the adrenogenital syndrome represents an inborn error of metabolism (15). In extension of this theme, it should be noted that it is tacitly assumed in endocrine studies that, despite a certain deviation consistent with biological results, a unit of hormone produces the same unit of response. This is apparently not so, for careful experiments by Young and his associates with different strains of guinea pigs show very considerable quantitative differences in the response to injected testosterone (77, 90) This result, too, should cause us to be less rigid in making distinctions between certain differences in hormone levels.

## III. ALTERED STEROID SECRETION IN ENDOCRINOPATHIES WITH MENTAL DISORDERS

The first association of endocrine dysfunction with mental disorder was probably Plattner's recognition of mental deficiency in cretins in 1600 The adult form was not identified for another 270 years, and the high incidence of psychoses clearly indicated in 1888 by the Committee of the Clinical Society of London (18) This association, neglected for many years, has been reemphasized by Asher (4) and well defined by Lidz (58). While the thyroid secretions are not of a steroid nature, being derived in part at least from tyrosine, thyroid deficiency is associated with a marked decrease in the excretion of 17-ketosteroids (1). It is not implied that this is the cause of the mental aberrations, but it indicates an association between thyroid function and steroid metabolism that has not been fully explored

The decreased steroid output in Addison's disease is clearly a direct consequence of the deficient adrenal cortex Electroencephalographic records show brain function is altered in that abnormally slow EEG waves occur (33, 34, 54, 89) A similar slowing of the EEG pattern has been found in adrenalectomized rats (8). Cortisone restores the

slow activity to normal (54, 89). In rats there can also be demonstrated a decrease in the electric shock seizure threshold following injections of cortisone, and an increase after deoxycorticosterone (48, 95). Clinically, there are frequent alterations in the personality (19, 33, 34, 85). Apathy and negativism are prominent in three-quarters of the cases, seclusiveness, depression, and irritability in about one-half, while paranoid states are seen in 5 to 10%. Without going into great detail, further salient points can be made The incidence of psychopathy shifts from a premorbid rate of 25 to 60% after the development of the disease (85); the mental aberrations may occur independently of any significantly identifiable electrolyte disturbance, cortisone, in small amounts, may not only lead to correction of the personality disturbance, but also to temporary exacerbation of the mental defect of psychotic proportions (24). Persistence of treatment fortunately leads to cure of the psychosis. An exception has been reported by Cumming and Kort (26). They found a psychotic Addisonian refractory to electroconvulsive therapy (E.C.T.) and to cortisone, but who recovered with E.C.T. after the cortisone. Whether or how the steroid facilitated a favorable response to E.C.T. cannot be decided, but such unique instances sometimes provide unexpected clues. It supports an opinion of Cohn *et al* (25) who, in 1951, alleged that cortisone enhanced the effects of E.C.T.

The increased steroid secretion occurring in Cushing's syndrome carries with it a high incidence of psychiatric symptoms of great diversity (19). Depression with retardation, agitation, anxiety, paranoid ideation, and hallucinations are all seen. Organic mental changes, such as memory defect, distortion, and confusion occur in half the severe cases Since the psychological changes may precede the physical, there is good evidence that it is not just a reactive phenomenon (19).

It is asserted by Bleuler (9) and Reiss (71), that there is no characteristic difference in the psychopathologic changes seen in Addison's or Cushing's syndrome The writer believes that there are similarities, but that the application of projective techniques to significant groups of such cases would reveal objective differences, which he thinks he has detected by clinical appraisal. Be that as it may, another point remains to be made, and that is, as Reiss remarks, that many such endocrinological disturbances do not show psychopathologic symptoms, and that therefore one must postulate a constitutional or personality predilection for those who do

within reason, but in the short space of a hundred years since it was first utilized for experimental purposes, it has changed mightily. Its wild cousin, as collected in Baltimore and studied by Richter (76), is a different animal Untamable, vicious, and suspicious, it would appear to bear these characteristics in its germ plasm, for the young, foster-mothered by tame laboratory types, grow up true to their wild forebears. Endocrinological characteristics of the wild strain include small pituitaries and enormous adrenals. While not endeavoring to suggest that the glandular morphology affects the behavior, the association is worth noting. The genetics has been neglected, and only now receives revived attention. It is of current interest that a recent paper provides evidence that the adrenogenital syndrome represents an inborn error of metabolism (15). *In extension of this theme, it should be noted that it is tacitly* assumed in endocrine studies that, despite a certain deviation consistent with biological results, a unit of hormone produces the same unit of response. This is apparently not so, for careful experiments by Young and his associates with different strains of guinea pigs show very considerable quantitative differences in the response to injected testosterone (77, 90) This result, too, should cause us to be less rigid in making distinctions between certain differences in hormone levels

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An understanding of the mental changes in hypercorticalism, whether due to disease or medication, would be most desirable. Quarton *et al.* (68) have published a masterly review of the explanatory hypotheses bearing on the subject of mental disturbances accompanying the administration of ACTH and cortisone. They concluded that there is no completely satisfactory explanation. Indeed, neither the mechanisms involved nor the meaning for psychiatry are as yet discernible. It would be of little profit to reiterate all the speculations put forward, but it is perhaps pertinent to refer briefly to some expressed by psychiatrists whose experience with "cortisone" psychoses has been intimate. Despite the report of cellular changes in the hypothalamus after high doses of ACTH (13), and other data of some relevance (68), Goolker and Schein (43) rightly emphasize the dearth of evidence for an anatomical or functional locus of action of the corticoids on the brain. They describe the effect of ACTH and cortisone as producing a "ready state" in their patients, marked by irritability, tension, and emotional lability, resembling only superficially the action of other cephalotropic drugs, like the benzedrine group They feel they can only discuss the action of these agents in terms of that function of the ego having to do with the perception of stimuli They did not consider it justifiable to ascribe the occasionally observed intrusion of instinctual drives into the clinical picture to the action of the hormonal agents In the alerted condition seen in these patients, they claim that ideational stimuli assumed great temporary influence in determining the direction in which affect would change. F. L. Engel (31) has emphasized that hormones may alter existing biochemical reactions, but do not initiate new ones. True as this may be, it does not mean that the psychic outcome may not differ

An increase in instinctual tension has been proposed by Fox and Gifford (39) as the best available explanation for their clinical observations of altered mental states with ACTH and cortisone. They say that the mobilization of energy for cellular work peculiar to these agents results in a disturbance in homeostatic equilibrium which leads to the increased instinctual tension They support this contention by reference to Kubie's discussion of instincts and homeostasis (56) and by drawing attention to the constant concurrence of three findings in their cases, namely, alterations in appetite, in sleep, and in motor activity. These fall into the category of instinctual activities and, as is well known, the hypothalamus plays an important role in their regulation In certain cases, e g , anorexia nervosa, the expected reaction was not seen, for refusal to eat and withdrawal into mutism and immobility occurred. Fox explains this negativis-

## IV. EXPERIMENTAL ELEVATION OF ADRENAL STEROID LEVELS AND CEREBRAL CHANGES

The occurrence of mental symptoms accompanying the use of cortisone and ACTH was a finding which intrigued and amazed the medical world four and five years ago At the height of the investigative exploration of these agents, it was not uncommon to hear that 70% of patients treated became euphoric and 5% psychotic (19, 42). In patients receiving ACTH therapy, the production of slow brain wave frequencies was reported (53). Pine *et al.* (66) found less frequent abnormalities, in fact only in 7.5%, and in 7 of 12 showing abnormal EEG pattern there was definite improvement. Quarton *et al.* (68) discuss these and other reports in critical detail, concluding that the EEG evidence is indecisive

Psychiatric interest aroused by early experiences with ACTH and cortisone seems now to have largely subsided. The euphorias, depressions, and psychoses which aroused so much interest (17, 39, 42, 43) are little heard of now. This may be, in part, due to a real decrease in incidence resulting from more cautious administration, but probably it also represents a lack of reporting Accompanying the early reports on the mental and emotional disorders elicited by ACTH and cortisone, a number of workers made an effort to explain the results on the basis of a preexisting personality predilection. Glaser (42) and Cleghorn (19) have both taken exception to this point of view, submitting that some of the cases becoming psychotic under treatment had previously had stable personalities, and others, allegedly schizoid, did not. This facet of the problem was specifically examined by Lewis and Fleminger (57) who treated with cortisone or ACTH 12 patients having a history of recent mental illness No major mental changes occurred. They concluded that predisposition to untoward mental symptoms with such treatment cannot be assumed on the basis of the previous neurotic personality or prior mental illness In the last three years, few papers have appeared on the subject. Last year Fleminger (37) described data which he believes represent a different effect of ACTH and cortisone, the former producing depressive symptoms, and the latter an elevation of mood The suggestion is put forward that a change in the relative proportion of androgens to corticosteroids may be the determining factor in the type of mental response elicited. Since this may vary from case to case, it is not surprising that other workers have not observed a consistent difference in the psychological picture produced by the two agents. Only serial observations in many cases could provide data for the testing of Fleminger's hypothesis.

chronically hospitalized schizophrenics to ACTH as judged by eosinophil and lymphocyte changes, which, they say, confirms the work of others. They also found that ketosteroid and corticoid excretion varied within the normal range They surmised that uniform findings will emerge only when a group of remarkably uniform schizophrenics is secured The findings of Bliss *et al* (10), comparing schizophrenics to normals with respect to the blood cellular and 17-hydroxycorticosteroid response to ACTH, do not contravert the results of the Worcester group as they allege, as Hoagland and Pincus (50) have been at pains to point out recently. Severe emotional disturbances do not appear to raise the blood level even as much as ACTH or even exercise (11).

In the detailed studies of Hoagland, Pincus, and their associates, certain differences in urinary excretion were found in patients compared to controls, at rest and as a result of ACTH injections or stress tests. At rest, the schizophrenic groups showed a significantly higher rate of excretion of water, 17-ketosteroids, sodium, and potassium, but lower values for "cortins" (neutral reducing formaldehydogenic and lipids) and inorganic phosphates Great care was taken in statistical analysis. These data imply an increase in adrenocortical function, as judged by 17-ketosteroids, and a decrease, as judged by cortin. A recent additional bit of information, provided by Hoagland and Pincus (50), which helps to understand the water and electrolyte difference, is to the effect that aldosterone is not found in the urine of the majority of chronic schizophrenics

The stress tests and ACTH injections indicated that the patients' adrenal responses were subnormal with respect to water, 17-ketosteroid, sodium, potassium, and uric acid excretion There was always an increase in phosphate excretion in the patients during the procedure, whereas in the controls it showed little change (51, 64, 65) There were some interesting differences between the two schizophrenic age groups studied In the 20- to 40-year group, 72% responded inadequately to ACTH (64, 65), while only 37% of the 40- to 60-year-olds failed to respond (51) The total response value for the three stress tests was about the same for the two age groups This looks as if the major defect in the older group is at the hypothalamic level or the pituitary, while that in the younger is at the adrenal cortex The younger group, they say, contained more acute cases which might have been exhausted

Other workers, e g., Faurbye *et al* (36) and Reiss *et al* (72) have found some psychotics to show an inadequate response to certain stresses or ACTH The results are reported in such a different way that it is dif-

tic response (38) as occurring "when the intensification of specific instinctual conflicts about eating and locomotion created intolerable anxiety and required an equivalent intensification in the neurotic inhibitions against these activities." In this latter paper, Fox also emphasizes the change in perception experienced by certain patients which, at times, assumed psychotic proportions. G. L. Engel (32) has submitted a critical appraisal of this thesis.

Mirsky *et al.* (62) have also discussed the interaction of hormones and the nervous system on cellular function, particularly as related to adaptive behavior. They found in monkeys, conditioned to press a bar in order to avoid the shock following a sustained tone, that extinction of the avoidance response (bar pressing) took months to disappear in controls after cessation of the primary reinforcement. However, ACTH-injected animals developed extinction of the avoidance response quickly. These experiments, they say, mean that the ACTH facilitated the loss of effectiveness of the anxiety-producing stimulus or loosening of the fixation of the traumatic memory, or, in other words, of the most recently acquired defense. This experimental approach warrants attention, drawing notice as it does to learning theory and behavior in these primates resembling denial seen in hypomanic states.

No papers of equivalent importance extending these observations or submitting more acceptable explanations have appeared in the last two or three years. It is hard to escape the conclusion that this once exciting area of investigation has subsided for lack of pertinent or relevant techniques available for enlightening the findings New approaches are needed and, so far, have not appeared.

## V. STEROID METABOLISM IN THE PSYCHOSES

Evidence of various kinds implies aberrations in steroid metabolism in psychotics. Hoagland and Pincus and their colleagues (46-49, 51, 64, 65), have described the effects of ACTH and three tests yielding stress responses of the adrenal cortex in psychotics and normals To deal with *the blood cell changes first, these constituents did not clearly differentiate* the two groups, though lymphocytes and eosinophils were lower in the 40- to 60-year-old group of patients than in the controls in the resting state (51, 64, 65). Altschule (2) also found eosinophils to be lower in schizophrenics These cells were not differently affected, to any significant extent, by ACTH or the stress tests in patients and in the controls, nor did the corticoids behave differently This result was essentially confirmed by Dickes *et al.* (29) in a study of the response of eleven

months are of interest when speaking of longitudinal studies. They found a low corticoid excretion coinciding with hypomanic phases. This is the opposite picture to that reported by Reiss (70, 73) whose group have found depression to be accompanied by a decrease in corticoids and a rise in $\beta$-hydroxy-17-ketosteroids.

Assay of the rate of excretion of neutral 17-ketosteroids in schizophrenics has not shown any difference compared to normals, according to Reiss and Stitch (75), though a different diurnal pattern may occur in the patients along with a greater fluctuation However, schizophrenics have been found to excrete a greater percentage of 3 $\beta$-hydroxy-17-ketosteroids, this observation made by the Worcester group (63), was confirmed by Werbin *et al.* (93). The latter found the mean percentage of $\beta$-hydroxy steroids excreted by patients to be almost twice that of a control group An increased $\beta$-ketosteroid excretion was observed by Reiss *et al* (73) in the depressive phase of manic depressive psychosis. In order to obtain more information of a qualitative nature Reiss and Stitch (75) used a partition chromatographic method on the urine of chronic noncatatonic schizophrenics. They found what they considered statistically different values for the excretion of various 17-ketosteroids in schizophrenics They state also that an abnormal excretion of androsterone occurs early in the acute phase of the disease. In catatonics, Hemphill and Reiss (45) have described an apparently causal relation between the excretion of the $\beta$-ketosteroid, dehydroisoandrosterone, and clinical improvement.

Catatonic schizophrenics have been distinguished from paranoids by Stevenson, Metcalf, and Hobbs (84) on the basis of their reacting differently to adrenaline and ACTH as judged by eosinophile counts The paranoids were hyporeactive while the catatonics behaved more like normals. Such findings may help explain conflicting reports in the literature.

## VI ADRENAL STEROID THERAPY IN PSYCHOSES

The early reports of positive mood changes accompanying the therapeutic use of ACTH and cortisone in organic disease, led us at the Allan Memorial Institute in 1950, to try ACTH in the treatment of severe depressions (23) In eight such cases there were but slight clinical signs of improvement during the first day or two after starting the drug, which was given for seven days or more. This is not considered to be a specific effect, and most of the cases subsequently improved with E.C.T. The only apparent psychological finding was an increase in tension in some

ficult to make a comparison, but the trend appears to be conclusive. Reiss *et al.* (72) point out that ACTH may elicit different degrees of adrenocortical response. For example, the eosinophil function may be normal but ketosteroids may not be mobilized, or uric acid metabolism only may be disturbed. Since glucose and electroconvulsive therapy did not produce responses of the same order as ACTH, Reiss feels this implies that the ACTH secreted by the pituitary is different. Time factors and strength of the stimulus could explain the differences found. Elsewhere, Reiss *et al.* (70, 73) have drawn attention to another aspect of steroid excretion in schizophrenia, namely, the large fluctuations which, from day to day, may amount to 200% They also say that prolonged ACTH injections may lead to adrenal exhaustion, accompanied by mental deterioration.

Faurbye *et al.* (36) point out that such observations as the above do not account for the fact that hypertrichosis occurs oftener in schizophrenic females than in normals, an incidence of 60 to 36% in the patients versus controls being found in their hospital Faurbye and his associates found in their careful study of 12 schizophrenics great variation in the patients' responses to ACTH and in the adrenaline test with respect to 17-ketosteroids, uric acid, potassium excretion, and eosinophil decrease These findings, they feel, indicate an adrenocortical deficiency in schizophrenia They say that this could be related to the schizoid-leptosome constitution, but it is not certain whether this is an original trait or whether stress leads to the insufficiency, or whether the inactivity of the patient during the psychosis or a complex metabolic disturbance is the cause or accompaniment of the psychosis They feel that the formation of highly active 17-ketosteroids may explain the hypertrichosis

Following up this work, Vestergaard *et al.* (91), have made a careful study of the excretion of combined neutral 17-ketosteroids in 18 chronic schizophrenics. They found that the total excretion pattern was such as reported by Reiss *et al* (74), but that the ratio of the morning to night excretion rate was not low in the patients, contrary to the findings by Hoagland *et al.* (49). They point out that spontaneous variability must be taken into account for studies involving response in terms of 17-ketosteroid excretion and that cross-sectional studies done without longitudinal observations may be misleading They were unable to find any correlation to mood but feel that endeavors to set up subgroups of patients, using a variety of criteria, would provide clues for biochemical correlations

Observations of Rizzo *et al* (78) on a cyclothymic patient over many

months are of interest when speaking of longitudinal studies. They found a low corticoid excretion coinciding with hypomanic phases. This is the opposite picture to that reported by Reiss (70, 73) whose group have found depression to be accompanied by a decrease in corticoids and a rise in $\beta$-hydroxy-17-ketosteroids.

Assay of the rate of excretion of neutral 17-ketosteroids in schizophrenics has not shown any difference compared to normals, according to Reiss and Stitch (75), though a different diurnal pattern may occur in the patients along with a greater fluctuation. However, schizophrenics have been found to excrete a greater percentage of 3 $\beta$-hydroxy-17-ketosteroids, this observation made by the Worcester group (63), was confirmed by Werbin *et al.* (93). The latter found the mean percentage of $\beta$-hydroxy steroids excreted by patients to be almost twice that of a control group An increased $\beta$-ketosteroid excretion was observed by Reiss *et al* (73) in the depressive phase of manic depressive psychosis. In order to obtain more information of a qualitative nature Reiss and Stitch (75) used a partition chromatographic method on the urine of chronic noncatatonic schizophrenics. They found what they considered statistically different values for the excretion of various 17-ketosteroids in schizophrenics. They state also that an abnormal excretion of androsterone occurs early in the acute phase of the disease In catatonics, Hemphill and Reiss (45) have described an apparently causal relation between the excretion of the $\beta$-ketosteroid, dehydroisoandrosterone, and clinical improvement.

Catatonic schizophrenics have been distinguished from paranoids by Stevenson, Metcalf, and Hobbs (84) on the basis of their reacting differently to adrenaline and ACTH as judged by eosinophile counts The paranoids were hyporeactive while the catatonics behaved more like normals Such findings may help explain conflicting reports in the literature.

## VI. ADRENAL STEROID THERAPY IN PSYCHOSES

The early reports of positive mood changes accompanying the therapeutic use of ACTH and cortisone in organic disease, led us at the Allan Memorial Institute in 1950, to try ACTH in the treatment of severe depressions (23) In eight such cases there were but slight clinical signs of improvement during the first day or two after starting the drug, which was given for seven days or more This is not considered to be a specific effect, and most of the cases subsequently improved with E.C T The only apparent psychological finding was an increase in tension in some

of the cases, while all showed marked physiological effects of the hormone.

The occurrence of severe psychological changes in patients with organic disease receiving cortisone (17) led Cohn and his associates (25) to try cortisone in the treatment of schizophrenics (18 schizophrenics: 10 paranoid, 6 catatonic, 2 simple). He reported much more favorable results than those obtained by subsequent workers (14, 41, 69). More recently, Polatin *et al.* (67) have reexamined the problem and treated nine nondeteriorated schizophrenic women with massive doses (500 mg. daily for 4–10 weeks). Three showed slight transient improvement; one did not change, five were made worse, showing a marked increase in anxiety and tension. In commenting on this, they say, interestingly enough, that "The effect was similar to that seen in some schizophrenics when given an intravenous amphetamine injection." They express mystification that only two of their cases showed pronounced evidence of hyperadrenalism despite the massive therapy. This may mean tissue insensitivity and is reminiscent of the thyroid insensitivity of some schizophrenics. What appears to be a positive result with cortisone in a periodic catatonic schizophrenic has been described by Gornall *et al.* (44) in 1953. The merit of their success lies more particularly in the careful analysis of the steroid excretion from which they derived the indication for the use of cortisone. More recently Wiedorn (94), who seems unaware of the above work, reported the treatment of acute catatonic excitement with large amounts of cortisone. Improvement took place concurrent with this therapy. The author suggests that there may be a difference in the response of acutely ill versus chronically ill patients.

## VII. ADRENAL MEDULLARY AND CORTEX RELATIONSHIPS

In order to understand the action of adrenocortical steroids, attention must be paid to an obvious gap in our knowledge, namely, the relation of the cortical to the medullary secretion (21). This area occupied my attention formerly (3, 22), but it is only since Levine's (40) demonstration of the dependence of the vasoconstrictor effect of noradrenaline on cortisone and allied compounds, that the field has begun to open up. Vogt (92) has recently reviewed with great cogency data of importance such as the simultaneity of the release of ACTH and adrenaline. In addition to this type of evidence, it has been found by Von Euler (35) and his group that hypophysectomy led to a decrease in the adrenaline content of the medulla and an increase in noradrenaline, while ACTH had the opposite effect. Von Euler (35) has also shown that ACTH de-

creased noradrenaline secretion in the urine of patients with rheumatoid arthritis Cortisone had the same effect. His group also found an inverse correlation between 17-ketosteroids and adrenaline. As this type of investigation extends, we may find evidence implicating adrenocortical hormones with catecholamine metabolism in the brain. An active area of research at present concerns the factor or factors, operative in the release of ACTH. My associate, Saffran, and his colleagues, have devised elegant techniques for studies in this area (79) Their work shows that there is a corticotropin-releasing factor to be found in the posterior pituitary. This has been shown to be distinct from vasopressin and oxytocin and it provides the strongest stimulant to the release of ACTH from the anterior pituitary so far investigated (80, 81). The release of this material from its stores seems to require noradrenaline.

## VIII. THE LYSERGIC ACID LINK TO ADRENOCORTICAL FUNCTION

Considerable work in recent years suggests that an aberration in adrenaline metabolism may play a part in the production of schizophrenia, an area reviewed by the writer recently (21) Lysergic acid diethylamide (LSD), has become a useful tool for the experimental production of a schizophrenic-like state. Its action is allegedly that of an antimetabolite. Hoagland *et al.* (52) extended the psychic parallel of the action of LSD to a biochemical level in their discovery that this agent produced a urinary excretion of phosphate as low in normals as that seen in schizophrenics Furthermore, when ACTH was given to the LSD-treated normals, there resulted a gross increase in phosphate excretion similar to that occurring in ACTH-treated schizophrenics, but not occurring in non-LSD-treated ACTH-injected normals This finding, they feel, suggests that LSD acts on enzyme systems which facilitate the binding of phosphate, and that an endogenous derivative of adrenaline metabolism may act in schizophrenics in the manner of LSD. They propose that the corticoids release the bound form of phosphate, accounting for the large output in schizophrenics and LSD-treated normal subjects. Finally, the LSD seems to stimulate the pituitary-adrenal system, leaving the adrenal somewhat unresponsive to ACTH as measured by excretion of 17-ketosteroids, sodium, and uric acid, resulting in a condition similar to that seen in the schizophrenics.

In closing I can only suggest what seem to me to be significant points for study First I would express a preference for relatively isolated physiological preparations as used by Marazzi and Hart (59) Further

studies of genetic differences in responses to hormones seem to be highly pertinent. I doubt whether one can obtain from a study of endocrine diseases anything more than a small return for an infinite amount of work. Longitudinal study of psychotics, well documented at the clinical level, though tedious, should offer a good return The use of various psychomimetic and other drugs and their influence on steroid metabolism is essential. A closing of the metabolic relationships between steroids and the catechol amines is a key operation. Finally, it is essential in the long run to be able to correlate our psychodynamic constructs with various other classes of biological data. In other words, we must try to bring the languages of the biochemist, physiologist, and psychiatrist together. Possibly that is too much because it looks like asking for a solution of the mind-body problem.

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## Discussion

CHAIRMAN H. HOAGLAND. This is a very extensive field that has been covered by Dr. Cleghorn's review and there are a number of people here who have worked on various facets of the problem. The paper is open for discussion

C G HARTMAN. Not in all animals does the adrenal cortex sit on top of the medulla

R A CLEGHORN In higher animals. I once went to a fish market at three o'clock in the morning for many consecutive days checking elasmobranch interrenal bodies

G S GORDAN I am greatly indebted to Dr Cleghorn for this and many other reviews Going back to the subject of psychoses induced by cortisone or cortisol, when cortisone was first introduced for its action in rheumatoid arthritis, the doses that were used were about 100 mg a day and the incidence of psychosis was very high As the clinician discovered that these doses invariably brought on a good degree of Cushing's disease medicamentosa, he reduced them About 1955 the doses were around 40 mg a day and the incidence of psychosis was very much less

The mechanism of this response of course is not at all known The electrolyte changes interested Dr Hoagland, I think it is pertinent to point out that in 1955 the Δ-1 compounds were introduced and although the doses that were used were by now very conservative, the incidence of psychosis promptly jumped I don't have accurate figures on this incidence I can only rely on what my friends in the pharmaceutical industry give me Those who are in a position to get reports tell me that the incidence of psychosis induced by the Δ-1 compounds is very much higher than that which was produced by cortisone even in the large doses which were used when it first came out This seems to indicate that this central nervous system action is not connected with the gross electrolyte changes that are produced by cortisone and cortisol

I should like to ask if you have any information about electrolyte changes with reference to these central nervous system responses?

R A CLEGHORN The only information I have is from the older literature and the few cases which I saw You will remember there was a case published by Ransohoff in which the psychosis could be brought on and cured by the administration of potassium When the potassium fell the psychosis came on, and it could be

eliminated by the administration of potassium. This occurred two or three times. It is the only case that I know in which this particular association was verifiably demonstrated

G. H. Glaser: I think we have some information. We were never able to correlate changes in serum electrolytes with psychotic manifestations. We even treated patients that developed the psychoses with potassium salts to no avail, and so it seemed that while this may well be a factor, it seems to be rather elusive The only thing that I can think of now is that the intracellular shifts in the brain might be significant. What we are measuring in the serum just is not reflecting changes in the brain Thus occurrence of psychoses with the newer Δ compounds of course is very intriguing and I have observed a few of these cases in the past year too The whole question of the nature of the development of these psychoses is one we have argued about a great deal in various other sessions I feel that the primary effect of these hormones is on the central nervous system The information which you have already heard this morning indicates that so definitely that it is hard to escape This is not just a psychological reaction to a hormone or to a stress situation which exists in *limbo* These hormones have a very definite effect on the metabolism and on the subsequent electrical activity of the nervous system, especially the brain This is seen in the material described by Dr Woodbury and ourselves in relationship to seizure states and the EEG and in the anesthetic effects. There are others. We noticed, for example, that reflex changes, such as spasticity can be diminished by these hormones. The neuromuscular relaxant effect that was alluded to this morning is a similar interesting reaction. This is paralleled by the worsening of myasthenia, diminution in myotonia, and lessening of fasciculations These may be more peripheral nervous system alterations. However, the nervous system changes are primary and the individual then reacts according to his personality and environment The psychotic content, the type of symptomatology that develops, then depends upon these interacting factors.

We were interested in the question of predisposition with regard to preexisting brain damage. The incidence of psychosis with these hormones is perhaps higher in certain diseases such as acute lupus which does produce brain damage However, in contrast to this, we treated experimentally a large number of patients with multiple sclerosis, these patients are often euphoric and the hormones had no effect on this preexisting euphoria It did not expand into a hypomanic state as we feared it might

Dr. Cleghorn mentioned that predisposed schizoid personalities did not necessarily develop psychoses when treated with steroids We observed one patient who had a severe paranoid psychosis and her psychotic manifestations changed into an acute catatonic state when we administered ACTH There are any number of reports now of individuals who by retrospective anamnesis were relatively normally adjusted and who later became psychotic during hormonal treatment At the present time I certainly agree with Dr. Cleghorn that we have reached a kind of impasse. We need a new methodology to approach the problem I hope the interaction at this session will give us some hints.

Chairman H Hoagland. I would like to comment about the material of ours to which Dr. Cleghorn referred. In this we speak of patients unresponsive to

ACTH This is a technical definition of ours which has sometimes been misunderstood By it we meant that the response of a certain total index of adrenal function involving electrolytes, 17-ketosteroids, and corticoid and lymphocyte responses did not exceed a certain magnitude which was statistically characteristic of the normal control group It did not mean that responsivity to ACTH in some of the components of the index was not normal

In the case of the corticoids, for example, in contrast to 17-ketosteroids we found no difference in the amounts excreted in response to ACTH by normal and by schizophrenic patients Our view has been that there is a difference in the metabolism in steroids in that precursors of the 17-ketosteroid group are somewhat different and there is some evidence for this from chromatography So I want to correct the view that we have said there was no response to ACTH in schizophrenics. Statistically as a group they are less responsive than the controls by some urinary measures of adrenal function and not by others Eosinophile and lymphocyte changes following administration of 25 mg of ACTH are not different in the two groups

The question of electrolytes is very interesting to us because of a decreased sodium output in chronic schizophrenics at rest that is characteristic of the schizophrenic population as a group, there is also a response to ACTH of reduced sodium and potassium output

This is in line with recent unpublished findings of Elmadjian of our laboratories who finds low or absent outputs of aldosterone in the chronic population of schizophrenics compared to normals although this is not true of the acute cases. These last tend to put out more, rather than less aldosterone in comparison to normals This ties in with the abnormal electrolyte picture of the chronic patients who at rest excrete much more sodium than normals and who are relatively unresponsive to ACTH in electrolyte excretion in a consistent way There is not enough aldosterone at the present time available to us to treat patients I hope we will be able to do that eventually and see what happens

Another possible role of steroids in psychoses is that some steroid or its metabolites may have a toxic action With this in mind, in collaboration with Charles Huggins of the University of Chicago, six schizophrenic patients were totally adrenalectomized and were studied either at the Billings Hospital in Chicago or at the Worcester State Hospital They were maintained on cortisone or hydrocortisone Unfortunately we were unable to find any consistent changes in the personalities following adrenalectomy We knew that patients who have been adrenalectomized and who are not schizophrenic and who are maintained on cortisone do not become psychotic or have abnormal symptoms Therefore, if one adrenalectomizes and maintains these people on cortisone it might be expected that the abnormal steroid substances, if present, would thus be eliminated and this might have therapeutic value But adrenalectomy did not produce significant improvement in the patients

It would seem that the abnormalities we have found in urinary studies of specific production of steroids in the schizophrenic group are probably a reflection of more fundamental disturbances in enzyme function and are probably not causal to the psychoses The fact that steroids are involved in some psychoses and that overdosage will produce psychosis in some persons and also that one

may get psychosis in Addisonians is puzzling, but it is perhaps secondary to other basic chemical disturbances.

Have you any additions to make to what I have said, Dr. Pincus?

G. PINCUS: I think that the problem of steroid metabolism isn't solved Every attempt at replacement therapy puts you at the mercy of the steroid you are using. So far in adrenalectomized patients the only way to maintain with any adequacy is either with cortisone or hydrocortisone and our studies in the schizophrenics and normals so far have not revealed any very significant differences in urinary metabolites when they are on replacement therapy compared to pre-adrenalectomy. So that unless there is some other source of abnormal steroid, I think that hypothesis can safely be eliminated. The more interesting feature is the possibility of some sort of servo mechanism between steroid and centers in the brain and this is going to take much more work.

R. A CLEGHORN· It seems to me, Mr. Chairman, we must remember that once certain neurotic or psychotic mechanisms have been set in motion they are very difficult to change. If a cerebral pattern of behavior has been set up it does not mean that if we withdraw a certain reinforcing agent it would be altered.

CHAIRMAN H HOAGLAND I agree.

D W WOOLLEY. I have been listening to many of these findings on schizophrenic patients Many at least can be related to an idea based on serotonin. I wonder if there was any evidence that cortisone, for example, affected the blood-brain barrier There is the belief that serotonin produced in the periphery does not get into the central nervous system, at least with any great efficiency. If cortisone, for example, were to reduce the blood-brain barrier, peripheral serotonin would get into it with greater efficiency

D M WOODBURY We have evidence that cortisone increases the chloride space of brain, and this effect might be due to an increase in permeability of the blood-brain barrier to chloride

D. W. WOOLLEY You would expect that from all the actions of cortisone it would do that

D. M. WOODBURY However, the cortisone might also have other effects. For example, Dr Ruth Geiger has shown that this steroid inhibits the growth of glial cells and fibroblasts in tissue cultures of gray matter If this action occurs *in vivo* the increase in chloride space of brain caused by cortisone could be due to this effect of the steroid on the glial tissue of brain and not to an increased permeability of the blood-brain barrier or brain cells to chloride

D W WOOLLEY My thinking revolved around the idea that the blood-brain barrier had as one of its purposes to protect the central nervous system from peripheral serotonin and adrenaline I wonder if this would fit some of the data.

CHAIRMAN H HOAGLAND Mark Mason, J R Bergen, Eric Block, and I (unpublished) have examined the cerebrospinal fluid, urine, and blood of dogs at intervals following intravenous or intra-arterial injection of $C^{14}$-labeled steroids together with nonradioactive steroid carriers In different experiments, labeled $\Delta^4$-androstenedione, cortisol, and testosterone were used in doses of from 30 to 100 mg per dog Carrier steroids of the injected substance and its principal metabolic products were added to the samples and a variety of chromatographic and chemical procedures were used to identify the steroids found associated

with radioactive fractions. These fractions in the cerebrospinal fluid appeared to be associated both with the originally injected steroid substance and with some of its metabolites Recrystallization of the chromatogram eluates from cerebrospinal fluid, however, resulted in such small quantities of steroid and such reduction in radioactivity that we were unable to prove conclusively that the activity was due to the specific steroid which had passed the blood-brain barrier Indications, however, were that the radioactivity did reflect the injected steroid and its metabolites in the cerebrospinal fluid. The blood-brain barrier is, however, very impermeable to these substances

D W. WOOLLEY· It is not necessary for them to pass, but only to reach and *change the barrier.*

CHAIRMAN H. HOAGLAND· Yes, it may be that modifications of electrolytes or of other nonsteroidal metabolites might be reflected in changes in brain function, if such agents resulting from the effects of the steroids were to pass the blood-brain barrier.

I. H PAGE I am still puzzled about the adrenal cortical hormones If they don't get into the brain, how do they control the threshold?

CHAIRMAN H HOAGLAND. They may, of course, enter the brain and be metabolized therein so as not to appear in the cerebrospinal fluid in appreciable amounts in the form of steroids. There is the possibility that they affect other processes in the systemic circulation in the body which have secondary effects on the brain There was a time when the question of whether changes in electrolytes in their own right as the result of the administration of DOC, for instance, was not perhaps related to the effect of this substance on the brain. I think that the steroids get into the brain, but that we did not get very much out in the cerebrospinal fluid. What do you think about it, Dr Woodbury?

D M. WOODBURY· Some recent observations from our laboratory are of interest in this connection We have studied the penetration of 4-$C^{14}$-cortisol into the various parts of the brain and other tissues of rats It was found that the volume of distribution of the radioactivity was 4% of wet brain weight for a period of from 5 minutes to 3 hours after intravenous injection As the plasma level decreased, the radioactivity in the brain decreased proportionally until at 3 to 4 hours after injection the concentration had decreased almost to zero. We have demonstrated previously that the extracellular space of the brain consists of two phases: a rapid phase, which is in rapid equilibrium with the plasma and constitutes about 4% of brain weight, and a slow phase, which makes up about 21% of brain weight. Thus it would seem that the radiocortisol is distributed in the rapid phase of the brain extracellular space during the first 3 hours after administration, and that it is in rapid equilibrium with the plasma radiocortisol After 4 hours, however, there was a secondary uptake of radioactivity by the brain This increase in radioactivity reached a maximum at 8 hours and *remained at this maximum* for 24 hours at which time the ratio of brain to plasma radioactivity was about 3 to 1. Thus the brain was able to concentrate to a considerable extent a radioactive product of cortisol These data definitely indicate that radiocortisol and/or its metabolites enter the brain Whether the effects of cortisol on electroshock seizure threshold are due to cortisol *per se* or to one of its products awaits further investigation We are now attempting to identify the radioactive product(s) in the brain

O. DIETHELM: In our studies of episodic confusional states, which we separate from schizophrenia, we found in one case a rather interesting low phosphate excretion before, and high calcium retention at the peak of each excitement which occurred about every 4 to 6 weeks. In another case we had indications but the findings were not clear These cases were studied on our metabolism floor for several months I think these observations bring out the point that findings may be bewildering if one does not study the same patient over a sufficient length of time I also would like to stress that one should not be too much impressed by the diagnosis of schizophrenia Schizophrenia is not a well-defined illness, but consists of types that can be broken up into several subgroups which in many ways are quite different.

The other point I want to mention refers to the remarks on sexual drive and aggression. In many psychiatric patients it is the aggression which produces the sexual drive, secondarily probably, just as frequently as the sexual drive produces the aggression I think these are two separate aspects which are linked but have not been investigated

R. W. RAWSON. I want to ask two questions The first one concerns the hypertrichosis that you said occurs in schizophrenics. I would appreciate it if you would define and describe for us this hypertrichosis The second question has to do with your reference to a phosphate retention seen in schizophrenic patients. What is the degree of this retention of phosphate and what is the effect of shock therapy or of adrenal stimulation on this unusual phosphate balance?

R. A CLEGHORN As far as hypertrichosis is concerned, obviously it can only be identified in females, as an extension, for example, of the pubic hair, as a development of areolar hair and of hair on the face

R W. RAWSON. Is there a growth of hair on the shoulders and back?

R A. CLEGHORN. I have not identified any cases in which it was markedly changed on the shoulders and back and I have not read the original papers to which Faurbye refers. I have seen one case of a girl who has had several episodes of a schizophrenic nature who has quite a bit of beard on the chin, and when she develops an episode the axillary and facial hair grows faster, according to her own observation

R. W. RAWSON: We have observed an hypertrichosis developing in young females subjected to ablation of the thyroid, in an attempt to induce function in distant metastases from cancers of the thyroid, and a disappearance of the hypertrichosis on restoration of these girls to a euthyroid state I wonder if the hypertrichosis observed in these patients might be related to some abnormalities of thyroid function.

R A CLEGHORN: I have seen one such case many years ago I don't know what the explanation of that is, I am sure

G. S. GORDAN: As endocrine consultant to a psychiatric hospital, I would like to offer still another explanation I think that areolar hirsutism is normal and that facial hirsutism in psychiatric patients of all types, not just schizophrenic patients, is merely a reflection of less cosmetic care to the normal facial hair which brings so many patients to endocrinologists

R. A. CLEGHORN: One of my colleagues says his observation is that there are fewer razor blades in mental hospitals

R W GERARD Dr Cleghorn discussed the effect of cortisone and ACTH as more or less equivalent Dr. Glaser did also I am concerned with the fact, I believe, that a rare complication of the administration of ACTH to man, in the doses used in treating rheumatoid arthritis, etc, is a massive transversemyelitis with degeneration of the cord—perhaps a dozen cases have been reported—but this complication has not been reported after cortisone Does this mean there is some independent action of ACTH aside from that via cortisone liberation?

R A CLEGHORN: Initially Rome and Braceland tried to differentiate between the types of pictures seen with these two agents I don't think other people agreed. More recently Fleminger gave a patient ACTH on one occasion and cortisone at another and thought he could distinguish between the types of pictures produced by the two agents Again I think that the evidence is not very good and as far as the transmyelinization business, I have no information on that, so I cannot answer you

G. PINCUS: I want to make one more comment about steroids. At a similar conference here a year ago I pointed out that the 17-ketosteroids, in the older age groups and in other persons with chronic diseases, on the average are low, actually not high, as compared to younger persons and that this whole situation appears to be the result of having "a chronic disease" Every chronic disease patient that we have studied puts out 17-ketosteroids at a level which is characteristic of a person many years older than himself, and this suggests the picture that is seen in chronic stresses generally, namely, the 17-ketosteroids are the most labile to chronic stress whereas the corticosteroids do not seem to be so affected When we studied the corticosteroids there was no such effect in terms of absolute output Furthermore, when we fractionated the urinary steroids what we found was that those ketosteroids which originated from the true adrenal corticosteroids did not show the effect of chronic disease, whereas the 11-deoxy-17-ketosteroids did This suggests that the picture of steroid imbalance is not unlikely, but what the repercussions of such an imbalance are is going to be very difficult to resolve because of the fact that the adrenal cortex produces certainly a minimum of three outstanding substances and probably a number of others That may be very important I think that our original work was very courageous and foolhardy in the clinical sense but, on the other hand, there were no other tools available to us and I think this more recent work does point out the fact that there is some effect which is nonspecific to schizophrenia, perhaps, but which may be a reflection of this disbalance which has occurred, say in the acute stage

# Influence of Adrenocortical Steroids on Brain Function and Metabolism[1]

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A previous report from this laboratory (21) has summarized the effects of adrenocortical steroids and adrenocorticotropic hormone (ACTH) on central nervous system excitability as measured by the electroshock seizure threshold technique (25). This summary emphasized the marked influence of adrenocortical steroids on brain excitability and the relation between their effects on excitability and on electrolyte metabolism. The importance of changes in brain intracellular Na concentration in relation to changes in brain excitability was emphasized and it was suggested that changes in brain K concentration were less important. In addition, evidence was presented that the adrenocortical steroids exert a regulatory influence on brain excitability. This regulatory function is operative only when changes in excitability occur, the adrenocortical hormones then act so as to restore normal brain excitability, regardless of the direction in which the deviation tends.

The present investigation is designed to contribute further knowledge concerning the regulatory effects of adrenocortical steroids on brain function and metabolism The following topics will be discussed: I. Effects of acute and chronic administration of adrenocortical steroids on electroshock seizure threshold (EST) and on the metabolism of brain electrolytes and amino acids. II. Influence of adrenocortical hormones and other agents that influence carbohydrate metabolism on postictal recovery. III. Modification of adrenocortical function by centrally acting drugs and the influence of such modification on the central response to these drugs

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## I. EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF ADRENOCORTICAL STEROIDS ON ELECTROSHOCK SEIZURE THRESHOLD, AND ON THE METABOLISM OF BRAIN ELECTROLYTES AND AMINO ACIDS

[illegible] administration of the various adrenocortical [illegible] threshold, have been studied [illegible] The results are summarized in Fig. 1 which is a composite graph [illegible] EST changes produced by six different adrenocortical steroids, each given chronically for 28 days The dose was 2 mg. per rat per day in all groups except in the deoxycorticosterone acetate (DCA)-treated animals, which received approximately 1.2 mg per day (six 15-mg. pellets implanted subcutaneously in each rat). DCA elevated the EST progressively with time, the rise amounted to 22% on the 25th day. The EST of rats treated with 11-deoxy-17-hydroxycorticosterone acetate (Substance S acetate) reached a maximum elevation of about 6% in 9 days and stayed at this level for the duration of the 28-day experiment. Corticosterone acetate (Compound B acetate) was tested for 16 days, after a transient decrease of 2 5% during the first two days, the EST returned to the control level and was not significantly altered during the rest of the 16-day period. At the dose level employed, this compound appears to have no sustained effect on brain excitability as measured by the EST 11-Dehydrocorticosterone acetate (Compound A acetate) decreased EST progressively until it reached minus 5% after 16 days, at which level it remained for the last 9 days of the experiment 17-Hydroxycorticosterone acetate (cortisol acetate, Compound F acetate) steadily lowered the EST until the level was minus 15% after 28 days The effect of 11-dehydro-17-hydroxycorticosterone acetate (cortisone acetate, Compound E acetate) on EST was more pronounced than that of cortisol acetate during the early phase of the experiment, whereas during the terminal portion the depression of EST paralleled that of cortisol and was also minus 15% at 28 days.

The changes in EST 6 hours after the administration of single doses of DCA (2 mg) and cortisol acetate (2 mg) to intact and adrenalectomized rats are shown in Fig 2. It is evident from an examination of this figure that single doses of these steroids exert a significant influence on EST. In addition, it is noted that the effect of DCA to elevate EST and of cortisol acetate to lower EST is significantly greater in the adrenalectomized than in the intact rats. Thus, the adrenalectomized rat responds

with a greater change in EST to the same dose of a steroid than does the intact animal, regardless of the direction in which the EST change occurs This observation would suggest that some product secreted by the adrenal cortex in response to the injected steroid acts on the brain in such a manner as to maintain the EST at a more normal level. This is probably corticosterone, although preliminary data indicate that aldosterone may have a similar action (21, see p. 35). These data indicate

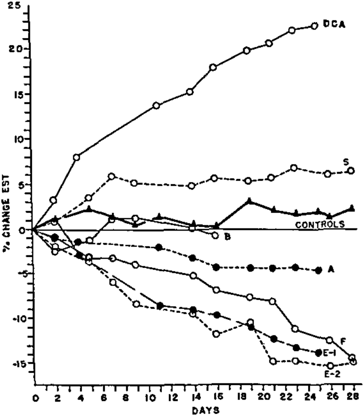


FIG 1 Effect of adrenocortical steroids on the electroshock seizure threshold (EST) of rats (20) Ordinate is % change in EST measured from control values at zero time Abscissa is time in days. DCA, deoxycorticosterone acetate, six 15-mg. pellets, S, 11-deoxy-17-hydroxycorticosterone acetate, 2 mg/day, B, corticosterone acetate, 2 mg/day, A, 11-dehydrocorticosterone acetate, 2 mg/day, F, 17-hydroxycorticosterone acetate, 2 mg/day, E-1 and E-2, 11-dehydro-17-hydroxycorticosterone acetate, 2 mg/day, two different experiments Controls are mean values for two different experiments See text for discussion

that an increase in plasma corticosterone concentration, such as occurs after administration of exogenous corticosterone or ACTH, tends to "normalize" EST. Such administration will counteract the EST-elevating effect of DCA and the EST-lowering effect of cortisol, without modifying EST in the normal rat. Therefore, secretion of adrenal steroids is capable of "normalizing" the excitability of the brain when it deviates from nor-

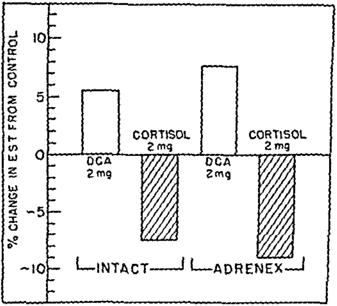


FIG 2 Effect of single subcutaneous doses of deoxycorticosterone acetate (DCA) and cortisol acetate on the electroshock seizure threshold (EST) of rats EST was determined 6 hours after injection of the steroids *Ordinate* is % change in EST measured from control values at zero time DCA, 2 mg, cortisol, 2 mg Adrenalectomized rats were maintained on 0 9% sodium chloride solution as drinking water See text for explanation.

mal, *regardless* of the direction of the deviation This subject will be further discussed in Section III.

Previous observations in this laboratory have shown that changes in the concentration of intracellular brain Na and/or in the ratio of extracellular to intracellular brain Na concentrations are associated with changes in brain excitability. Decreased intracellular brain Na concentration and/or increased brain Na ratio (e.g., such as occurs after thyroidectomy or hypophysectomy, or after chronic treatment with diphenylhydantoin [Dilantin] or DCA) results in decreased brain excitability (in-

creased EST). Increased intracellular brain Na concentration and/or decreased brain Na ratio (e.g, such as occurs after adrenalectomy or pancreatectomy, or after treatment with thyroxine, triiodothyronine, insulin, or alloxan) results in increased brain excitability (decreased EST). Because of these facts, the effects of single doses of DCA and cortisol acetate (as opposed to the chronic treatment previously described) on elec-

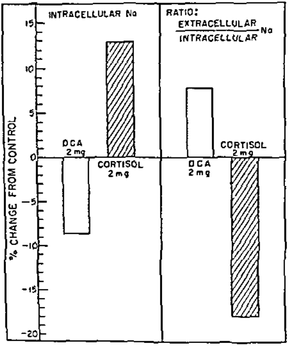


Fig 3. Effect of deoxycorticosterone acetate (DCA) (2 mg) and cortisol acetate (2 mg) on intracellular brain Na concentration and ratio of extracellular to intracellular brain Na concentration in adrenalectomized rats Rats were sacrificed for electrolyte determinations 6 hours after subcutaneous administration of a single dose of the appropriate steroid Ordinate is % change in concentration from that of the corresponding adrenalectomized untreated group. Intracellular calculations are based on chloride space as a measure of extracellular fluid volume of the brain See text for explanation

trolyte metabolism of the brain were studied in adrenalectomized rats. The effects on Na metabolism are shown in Fig 3. DCA decreased brain intracellular Na concentration, increased the ratio of extracellular to intracellular brain Na, and increased EST, in contrast, cortisol increased brain intracellular Na concentration, decreased the brain Na ratio, and

decreased EST (cf. Figs. 2 and 3). Acute administration of DCA thus alters brain electrolyte composition in the same manner as does chronic DCA treatment, and the alterations correlate well with the observed changes in EST. Acute administration of cortisol also produces changes in brain electrolytes which correlate with the observed changes in EST. However, after chronic cortisol treatment, EST appears to vary independently of brain intracellular Na concentration (19, 21). In this particular

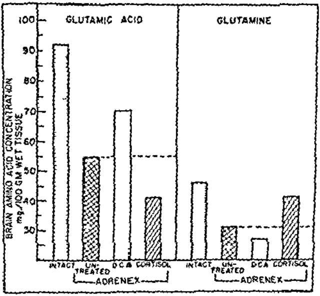


FIG. 4 Effect of single subcutaneous doses of deoxycorticosterone acetate (DCA) (2 mg ) and cortisol acetate (2 mg ) on free glutamic acid and glutamine concentrations in brain of intact and adrenalectomized rats The animals were sacrificed 6 hours after injection of the appropriate steroid Ordinate is brain amino acid concentration in mg /100 gm wet tissue See text for explanation

situation, EST may well be modified by the shift of hydrogen ions across cell membranes which occurs during chronic cortisol administration and in Cushing's disease (11, 14). Research on this problem is in progress

In summary, a reasonable explanation of these data is that the primary effects of DCA and cortisol on brain excitability are related to their influence on active Na transport in the brain. According to this view, DCA acts like Dilantin, which has previously been shown to stimulate active transport of Na out of brain cells (22) and by this process to decrease brain excitability. Cortisol, in contrast, inhibits active Na transport and

thereby causes an increase in both intracellular brain Na concentration and brain excitability.

The influence of single doses of DCA and cortisol on free amino acid metabolism in the brain of adrenalectomized rats was studied on the basis of the observations that: (a) adrenocortical steroids probably influence excitability by an action on active transport of electrolytes, (b) active transport of ions requires energy, (c) Terner *et al.* (15) have implicated glutamic acid and glutamine in transport of K by brain cells,

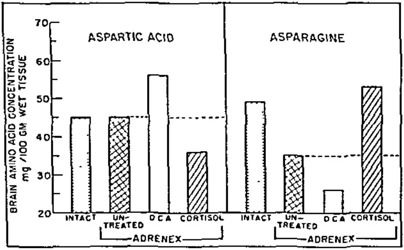


FIG 5 Effect of a single subcutaneous dose of deoxycorticosterone acetate (DCA) (2 mg) and cortisol acetate (2 mg) on free aspartic acid and asparagine concentrations in brain of intact and adrenalectomized rats The animals were sacrificed 6 hours after injection of the appropriate steroid Ordinate is brain amino acid concentration in mg./100 gm. wet tissue. See text for explanation.

and (d) adrenocortical steroids are known to alter profoundly the amino acid composition of various tissues (1). The important results are presented in Figs 4 and 5, in which the effects of DCA and cortisol on the concentrations of glutamic acid, glutamine, aspartic acid, and asparagine in brain are presented The steroids were given in single doses of 2 mg per rat, the animals were sacrificed 6 hours later, and the brains were removed for amino acid analysis by the technique of two-dimensional paper chromatography Adrenalectomy itself decreased the concentration of all 22 amino acids studied, with the notable exceptions of γ-aminobutyric acid which was increased, and of aspartic acid which was

unchanged. Both of these amino acids can be formed from glutamic acid. The decreases in free amino acid concentrations are compatible with the increased incorporation of free amino acids into proteins which occurs in adrenal insufficiency. The effects of cortisol and DCA on amino acids other than glutamic acid, glutamine, aspartic acid, and asparagine are compatible with the known influence of adrenocortical steroids on protein metabolism. However, the amino acid changes presented in Figs. 4 and 5 are unrelated to the predicted influence of these steroids on protein metabolism and appear to be more closely related to their effects on brain excitability. DCA increased the brain concentrations of glutamic and aspartic acids and decreased those of glutamine and asparagine. Cortisol, in contrast, decreased the concentrations of the free acids and increased the concentrations of the amides. Thus DCA and cortisol have opposite effects on brain excitability, on brain Na concentration, and on the metabolism of those amino acids that have previously been implicated in electrolyte transport in brain (15). The conversion of glutamic acid to glutamine and of aspartic acid to asparagine requires energy. Since cortisol may provide energy by enhancing protein catabolism, it is conceivable that the increased formation of glutamine in the brain is a result of increased availability of high energy bonds. DCA on the other hand apparently decreases the available energy and stops or reverses the conversion of the free acids to the amides It is of interest that Gordan *et al.* (3) have observed that amidation of glutamic acid in human brain was prevented or reversed by deoxycorticosterone glycoside (DCG). The data presented in this paper agree with the observations of Gordan *et al.* who postulated that DCG inhibits the amidation by inhibiting the oxidative reactions which supply the necessary energy. DCG also decreases the $Qo_2$ of brain (4, 6).

Terner *et al.* (15) have proposed that glutamic acid plays a role in active K transport in brain but they have not ruled out the possibility that Na rather than K is the actively transported ion The present studies which show a correlation between changes in brain excitability, Na metabolism, and glutamic and aspartic acid metabolism also suggest a role of these amino acids in electrolyte transport. It is likely that Na rather than K is involved, since the effect of DCA on Na metabolism in brain is opposite to that of cortisol, whereas the two steroids have the same effect on K metabolism. Further experiments to elucidate the role of these amino acids in active ion transport are clearly indicated.

Since DCA is not a normal secretory product of the adrenal cortex, it was of interest to compare the effects of aldosterone, the normal electro-

lyte-regulating hormone of the adrenal cortex, with those of DCA on EST. The results are shown in Fig. 6. EST measurements were made on aldosterone-treated and DCA-treated intact and adrenalectomized mice given water to drink. The doses used were equipotent with respect to Na retention. DCA elevated EST (15%) in intact animals and did so to an even greater extent (19%) in adrenalectomized mice. In contrast, aldosterone slightly decreased EST (7%), in the intact animals, but slightly elevated EST in adrenalectomized mice (4%). Adrenalectomy

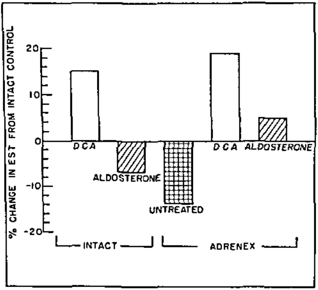


FIG 6 Effect of deoxycorticosterone acetate and aldosterone on electroshock seizure threshold (EST) of intact and adrenalectomized mice Ordinate is % change in EST from that of intact control mice DCA was administered in a dose of 0 5 mg/day for 3 days, aldosterone, in a dose of 20 µg/day for 3 days EST was determined on the afternoon of the third day. See text for explanation

itself lowered EST by 14% Thus, in the 3-day period of observation, aldosterone, in equivalent Na-retaining doses, has much less effect than does DCA on EST of both intact and adrenalectomized mice. The actions of aldosterone under these conditions are more like those of corticosterone, which it resembles structurally, than those of DCA, this suggests that its effects on the brain are related more to its corticosterone-like structure than to its electrolyte-retaining potency. These observations would suggest that aldosterone has a physiological role in the regulation of brain excitability.

## II. INFLUENCE OF ADRENOCORTICAL HORMONES AND OTHER AGENTS WHICH INFLUENCE CARBOHYDRATE METABOLISM ON POSTICTAL RECOVERY

Data previously presented from this laboratory have shown that the excitable process in brain is associated with transmembrane electrolyte shifts. It is also known that the excitable process is associated with a depletion of glucose, the chief source of energy for brain metabolism. The recovery process, which restores normal function following excitation, requires energy. In view of these facts, it was of interest to study the effects of adrenocortical steroids, and other substances which influence carbohydrate metabolism, on the rate of recovery of rats from maximal electroshock seizures and, in addition, to see if a relation exists between the effects of these substances on EST and their influence on recovery (18). The results are presented in Fig. 7. The recovery time ($RT_{50}$), which is defined in the legend to Fig 7, was used as a measure of duration of postictal depression. As illustrated in Fig. 7A, DCA elevated EST, cortisol and insulin lowered EST, and glucagon had no effect The influence of these same substances on $RT_{50}$ is depicted in Fig 7B, DCA had no effect, whereas cortisol and glucagon decreased and insulin markedly increased $RT_{50}$. These changes in $RT_{50}$ are unrelated to the changes in EST, but are inversely related to alterations in blood sugar level (Fig. 7C). Thus DCA, which did not alter $RT_{50}$, had no effect on blood sugar, cortisol and glucagon, which decreased $RT_{50}$, increased blood sugar, and insulin, which caused marked postictal depression, decreased blood sugar It may be concluded that whereas the excitable process is intimately associated with alterations in the ratio of extracellular to intracellular Na concentration in brain, the recovery process is intimately associated with changes in carbohydrate metabolism, particularly in blood glucose Glucose is the principal energy substrate for brain metabolism and, since glycogen stores in brain are small, the sugar in the blood is the chief source of brain glucose Therefore, any procedure or substance which influences blood sugar level will affect the recovery process provided the agent does not also interfere with the utilization of glucose by the brain Further studies on the effects of these substances on glucose utilization by brain in relation to the recovery process will be of interest

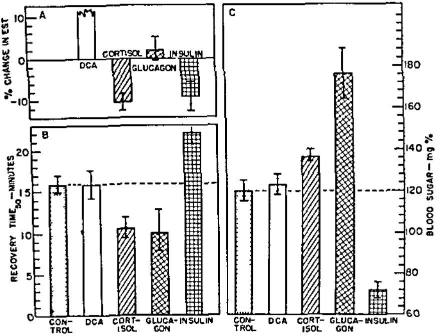


FIG 7 Relation between recovery time, electroshock seizure threshold, and blood sugar concentration in rats treated with various agents A Effect of deoxycorticosterone acetate (DCA), cortisol acetate, glucagon, and insulin on electroshock seizure threshold (EST) Ordinate is % change in EST referred to control values (zero baseline) before treatment Vertical bracketed lines represent standard error ( ... luration of depression follow ... the Recovery time$_{50}$ ($RT_{50}$ ... fined as the time required, after an initial maximal electroshock seizure (MES), for 50% of animals to recover to the extent that a second MES can be elicited Vertical bracketed lines are 95% confidence limits C Effect of the same agents as in A and B on blood sugar concentration Ordinate is blood sugar concentration in mg/100 ml blood Vertical bracketed lines represent standard error of the mean

The following doses and duration of treatment were used DCA, 1 5 mg/100 gm body weight, subcutaneously, daily for 12 days, cortisol acetate, 1 5 mg/100 gm, subcutaneously, daily for 12 days, glucagon, 50 µg/100 gm, intravenously in a single injection, measurements were made 45 minutes after administration Insulin, 10 Units/100 gm, subcutaneously in a single injection, measurements were made 2 hours after administration See text for discussion

## III. MODIFICATION OF ADRENOCORTICAL FUNCTION BY CENTRALLY ACTING DRUGS AND THE INFLUENCE OF SUCH MODIFICATION ON THE CENTRAL RESPONSE TO THESE DRUGS

The concept has been developed (see 21, and Section I of this paper) that the adrenocortical steroids act to "normalize" brain excitability. Recent workers [see discussion by Harris (5)] have demonstrated that the release of ACTH from the adenohypophysis is under the control of the central nervous system. The hypothalamus, which continually receives excitatory and inhibitory impulses from higher and lower centers, integrates these impulses and, via nervous pathways and the hypophyseal portal system, regulates the release of ACTH from the adenohypophysis

In addition, it has been known for some time that a single dose of Dilantin in rats does not appreciably elevate the electroshock seizure threshold, although the drug is very potent in abolishing the tonic extensor phase of the maximal (tonic-clonic) seizure pattern (an index of anticonvulsant potency). Other clinically effective anticonvulsant drugs, such as 5,5-diphenyltetrahydroglyoxaline-4-one (SKF 2599), phenobarbital, and trimethadione, are capable of elevating EST as well as abolishing the tonic extensor component of the maximal electroshock seizure. Previous experiments (2, 12, 20, 21) have suggested that Dilantin has an action on the adenohypophyseal-adrenocortical system.

For these reasons, the effects of centrally acting drugs on brain excitability were studied in order to answer the following questions. (a) Do such drugs affect adrenocortical function through their central actions? (b) If adrenocortical function is influenced, does the resultant enhanced or decreased secretion of steroids influence the central response of the agents being tested?

The following agents, all of which have central nervous system effects, were tested: Dilantin, a clinically effective anticonvulsant drug, SKF 2599, a congener which differs from Dilantin in that it markedly elevates the electroshock seizure threshold of intact animals in single doses, carbon dioxide, low and high concentrations of oxygen, and thyroxine The results are presented in Figs 8 through 15.

In Fig. 8, the influence of single doses of Dilantin and SKF 2599 on the electroshock seizure threshold of intact, adrenalectomized, and hypophysectomized rats is depicted At the time of peak effect of Dilantin (6 hours), only a small increase in EST was noted in intact animals, but a pronounced and essentially equivalent elevation was observed in ad-

renalectomized and hypophysectomized groups. SKF 2599 elevated EST to the same extent in intact, adrenalectomized, and hypophysectomized animals.

In order to determine whether the increase in EST induced by single injections of Dilantin in adrenalectomized rats could be prevented by adrenocortical steroids, the following experiment was performed. Intact and adrenalectomized rats were divided into two groups, one group each of adrenalectomized and intact rats received a single dose of 40 mg./kg. of Dilantin and the remaining two groups received the same

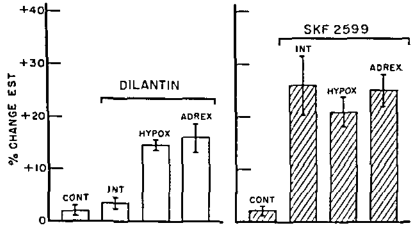


FIG. 8 Effect of single doses of diphenylhydantoin (Dilantin) and 5,5-diphenyltetrahydroglyoxaline-4-one (SKF 2599) on electroshock seizure threshold (EST) of intact, hypophysectomized, and adrenalectomized rats Ordinate is % change in EST referred to control (cont) values (zero baseline) before treatment. Dilantin was given in a dose of 40 mg/kg, subcutaneously, and SKF 2599 in a dose of 1000 mg/kg, subcutaneously. EST was determined 6 hours after drug injection Vertical bracketed lines represent standard error of the mean Int, intact group See text for explanation

dose of Dilantin plus 4 mg cortisone. The thresholds determined at the end of 6 hours are presented in Fig 9 Dilantin increased EST to a greater extent in adrenalectomized than in intact animals. Cortisone not only prevented the increase in EST induced by Dilantin, but caused a slight although insignificant decrease in EST in the intact rats and an appreciable decrease in EST in the adrenalectomized group It can be concluded from these experiments that, in intact rats, the injected adrenocortical steroids as well as those released by the Dilantin-induced stimu-

lation of the pituitary-adrenal system antagonize not only the EST-elevating properties of Dilantin but, as evident in the group given Dilantin plus cortisone, they also antagonize the cortisone-induced decrease in EST. Therefore, a product of the adrenal cortex (probably corticosterone or aldosterone) exerts a regulatory influence on brain excitability.

The effects of single doses of Dilantin and SKF 2599 on adrenal ascorbic acid and deposition of glycogen in liver, muscle, and brain were

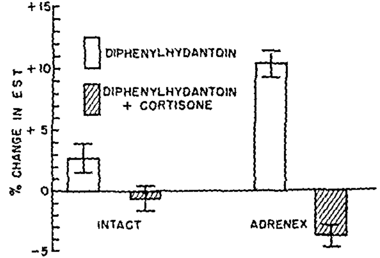


FIG. 9 Effect of diphenylhydantoin (Dilantin), alone and in combination with cortisone (4 mg), on electroshock seizure threshold (EST) of intact and adrenalectomized rats (21) Ordinate is % change in EST Vertical bracketed lines represent the standard error of the mean for each test group EST measurements were made 6 hours after the subcutaneous injection of 40 mg/kg Dilantin See text for discussion.

examined in order to assess the influence of these drugs on the pituitary-adrenal system. The results are presented in Fig 10. The concentration of adrenal ascorbic acid was decreased one hour after injection of Dilantin, SKF 2599, however, had no such effect. Hypophysectomized rats treated with Dilantin did not exhibit a drop in adrenal ascorbic acid concentration when examined one hour after injection of the drug. The concentration of glycogen in liver, muscle, and brain of intact rats was increased by Dilantin, but unaffected by SKF 2599. Glycogen determinations were made 4 hours after drug administration, previously determined

to be the time of peak effect of Dilantin on liver glycogen (21). Thus single doses of Dilantin, but not of SKF 2599, stimulate the pituitary-adrenal system, and the released steroids diminish the EST-elevating effect of Dilantin Adrenalectomy or hypophysectomy abolishes the pi-

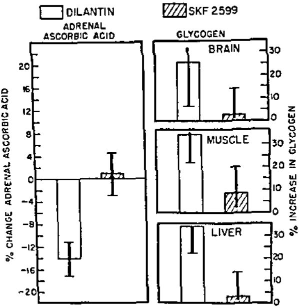


FIG 10. Effect of single doses of Dilantin and SKF 2599 on adrenal ascorbic acid, and on brain, liver, and muscle glycogen. Ordinate on left is % change in adrenal ascorbic acid concentration referred to the untreated control group Ordinate on right is % icrease in glycogen concentration referred to the untreated control rats. Vertical bracketed lines represent standard error of the mean Dilantin, 40 mg./kg., subcutaneously SKF 2599, 1000 mg./kg (ascorbic acid experiment), and 150 mg./kg (glycogen experiment), subcutaneously Animals were sacrificed for ascorbic acid determinations 1 hour after drug administration, and for glycogen determinations 4 hours after drug injection See text for explanation

tuitary-adrenal stimulating action of Dilantin and allows the full EST-elevating effect of the drug to occur

The observation that Dilantin stimulates the pituitary-adrenal system in single doses, whereas SKF 2599 does not, made it essential to determine whether the stimulating effect persists on chronic administration and, if so, whether the threshold-elevating effect is still modified by the released steroids Therefore, the influence of chronic administration of

Dilantin and SKF 2599 on EST of intact, adrenalectomized, and hypophysectomized rats was measured, the results are presented in Figs. 11 and 12. Dilantin (Fig. 11), given in a dose of 40 mg./kg./day for a period of 6 days, increased the EST by 20% in intact, 40% in adrenalectomized, and 48% in hypophysectomized rats. SKF 2599 (Fig. 12), given in a dose of 150 mg /kg./day for 6 days, increased the EST by 30% in all

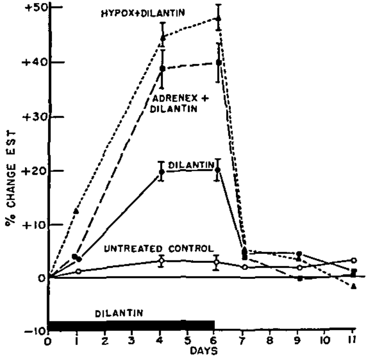


FIG 11. Effect of chronic administration of Dilantin (40 mg /kg /day for 6 days) on electroshock seizure threshold (EST) of intact, adrenalectomized, and hypophysectomized rats Ordinate is % change in EST from pretreatment values Abscissa is time in days Solid black bar on abscissa indicates duration of Dilantin treatment Vertical bracketed lines represent standard error of mean See text for explanation

three groups. Thus Dilantin exerts its pituitary-adrenal stimulatory effect during the entire period of its administration, whereas SKF 2599 has no such effect at any time

The small but statistically significant greater elevation in EST produced by Dilantin in hypophysectomized as compared with adrenalectomized rats may indicate that Dilantin, in addition to its adrenocortical stimulatory action, may also stimulate the pituitary-thyroid system to re-

lease thyroxine, since thyroxine is known to increase brain excitability (21). Preliminary experiments indicate that Dilantin does indeed stimulate the release of thyroid hormone In summary, then, it seems likely that Dilantin activates the pituitary-adrenal and the pituitary-thyroid systems and that the hormones released by such activation tend to counteract the anticonvulsant effect of the drug

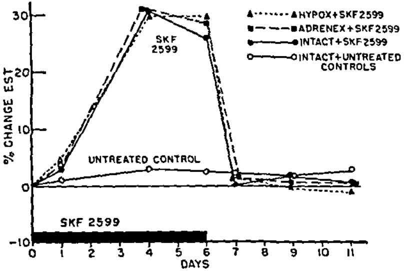


FIG 12 Effect of chronic administration of SKF 2599 (150 mg/kg/day for 6 days) on electroshock seizure threshold (EST) of intact, adrenalectomized, and hypophysectomized rats Ordinate is % change in EST from pretreatment values. Abscissa is time in days Solid black bar on abscissa indicates duration of SKF 2599 treatment See text for explanation

That chronic administration of Dilantin and SKF 2599 influences the activity of the pituitary-adrenal system is illustrated in Fig. 13 Both Dilantin and SKF 2599 (given for a period of 12 days) increased the size of the adrenals, but only Dilantin decreased thymic weight. The concentrations of steroids in the blood were determined by the fluorometric method of Sweat (13). Both Dilantin and SKF 2599 increased the concentration of corticosterone in the blood. Although Dilantin did not alter the level of cortisol, SKF 2599 caused a significant decrease The corticosterone/cortisol (B/F) ratio was thus higher in the SKF 2599-treated than in the Dilantin-treated group. Since only Dilantin produced a decrease in thymic weight and since cortisol is more potent in causing

thymic atrophy, the higher proportion of cortisol in the blood of Dilantin-treated rats resulted in thymic atrophy and antagonism of the EST-elevating action of Dilantin. Since single doses of corticosterone can lower an acutely elevated electroshock seizure threshold (21), it is necessary to explain why the markedly enhanced amount of corticosterone observed in the plasma of rats chronically treated with SKF 2599 does not prevent this drug from elevating EST. Although a firm answer is not yet available, it is likely that the alteration in B/F ratio induced by SKF

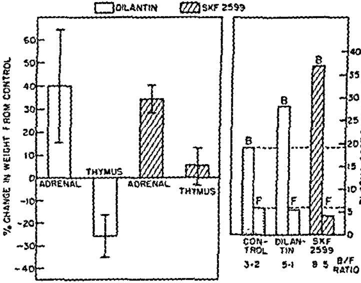


FIG. 13 Influence of chronic administration of Dilantin and SKF 2599 on adrenal and thymus weights and on plasma corticosteroid levels. Ordinate on left is % change in organ weight referred to control (zero baseline) values. Ordinate on right is plasma corticosteroid level in μg. %. Vertical bracketed lines represent standard error of the mean. Dilantin, 40 mg./kg., twice daily for 12 days. SKF 2599, 150 mg./kg., twice daily for 12 days. B, corticosterone, F, cortisol. See text for discussion.

2599 may account for the findings observed. The 30% reduction in concentration of cortisol, the steroid most active in lowering EST, permits SKF 2599 to exert its full threshold-raising effect on the central nervous system. The concurrent high concentration of corticosterone apparently does not prevent the increase in EST, which is in agreement with the results presented in Fig. 1

In summary of these results, SKF 2599 caused an increase in adrenal weight and corticosterone output, and a decrease in plasma cortisol level;

Dilantin also caused an increase in adrenal weight and corticosterone output, but did not decrease the plasma cortisol level. Some preliminary data indicate that the plasma level of cortisol in adrenocortical extract (ACE)-maintained adrenalectomized rats is markedly reduced by Dilantin, whereas that of corticosterone is unaffected In contrast, another stress procedure, such as electroshock, lowers the plasma levels of both cortisol and corticosterone in adrenalectomized animals maintained on ACE Since the levels of cortisol in intact rats treated chronically with Dilantin are unchanged, the results suggest that this anticonvulsant enhances secretion of cortisol by the adrenal

Previous experiments have demonstrated that thyroxine decreases electroshock seizure threshold (17, 21, 23). It has also been demonstrated that thyroxine increases adrenal weight, depletes adrenal ascorbic acid, and causes deposition of glycogen in the liver (16). Because of these observations and the fact that adrenal steroids markedly influence brain excitability, the effects of thyroxine on electroshock seizure threshold were studied in intact, adrenalectomized, and ACE-maintained adrenalectomized rats The results are shown in Fig 14 Thyroxine decreased the EST by 23% in intact animals, but by only 13% in adrenalectomized rats and by only 3% in adrenalectomized rats maintained on ACE. It was considered possible that the failure of thyroxine to lower EST as much in adrenalectomized as in intact rats was due to the lack of even a minimal amount of adrenal steroids and to the consequent decreased sensitivity of the brain to thyroxine This possibility is in accordance with the so-called "permissive" effect of adrenocortical steroids, as described by Ingle (7). If this proposal is correct, then the maintenance doses of adrenal steroids given to the adrenalectomized thyroxine-treated rats should have restored the normal response to thyroxine, and the EST in these rats should have been markedly decreased But this did not occur Instead, the ACE prevented the usual moderate reduction in EST which thyroxine induces in adrenalectomized rats, indeed, ACE abolished the thyroxine-induced lowering of EST, an exhibition of its "normalizing" effect. These results indicate that the adrenal cortex actively participates in the response of the central nervous system to thyroxine. Approximately half of the effect of large doses of thyroxine on the nervous system is the result of adrenocortical stimulation, the other half is the result of a direct stimulatory action of thyroxine itself on the brain.

Finally, as another example of the influence of centrally acting agents on the pituitary-adrenal system, the effects of carbon dioxide, hypoxia,

and hyperoxia on EST have been measured in intact and adrenalectomized mice. (Both carbon dioxide (10) and low oxygen concentrations [see review by Sayers (9)] have been shown by others to activate the adenohypophyseal-adrenocortical system.) The results of our experiments are illustrated in Fig. 15. Inhalation of 12 5% carbon dioxide increased EST to the same extent in intact and adrenalectomized mice.

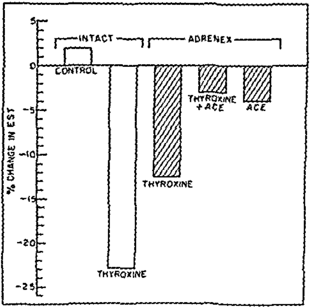


FIG 14 Effect of thyroxine on electroshock seizure threshold (EST) in intact rats, and in adrenalectomized rats with and without adrenocortical extract (ACE). Ordinate is % change in EST referred to control values prior to treatment. EST measurements were made 12 days after beginning of treatment The following doses were used· l-thyroxine, 40 μg /100 gm body weight/day for 12 days; ACE, 0 04 ml./100 gm /day for first 8 days, and 0 2 ml./100 gm./day for the last 4 days of the experiment. Adrenalectomized control rats received 0 2 ml /100 gm /day for the last 4 days of the experiment only See text for explanation.

However, inhalation of 30% carbon dioxide increased the EST to a significantly greater extent in the adrenalectomized than in the intact animals. Inhalation of 10% oxygen resulted in a greater decrease in EST in adrenalectomized mice, whereas inhalation of 75% oxygen increased EST to a greater extent in the intact mice It is concluded from these results that the corticosteroids released as a result of exposure of intact animals to high concentrations of carbon dioxide tend to decrease EST,

and in this respect carbon dioxide resembles Dilantin and thyroxine. Hypoxia and hyperoxia, in contrast, tend to release corticosteroids which increase EST in intact mice. Since recent data (8) suggest that aldosterone secretion may in part be regulated by the central nervous system, and also because this steroid can elevate EST under certain experimental conditions, it is necessary to entertain the view that hypoxia and hyperoxia can influence the central nervous system in a manner which results in the release of aldosterone from the adrenal Investigations to test this possibility are being carried out in our laboratory. In any event, it is evident that the central nervous system is involved in the control of adrenocortical steroids which can in turn modify the excitability of the brain

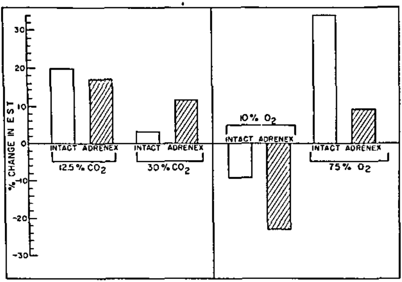


FIG 15 Effect of carbon dioxide, hypoxia, and hyperoxia on electroshock seizure threshold (EST) of intact and adrenalectomized mice. Ordinate is % change in EST from intact control animals The mice were exposed to the various gas mixtures for a period of 15 minutes prior to EST measurements See text for explanation.

## IV. SUMMARY AND CONCLUSIONS

An attempt has been made in this paper to present some of the interrelations between the cerebral cortex and the adrenal cortex with respect to brain excitability (as measured by electroshock seizure threshold),

postictal depression, brain electrolytes, and brain carbohydrate and amino acid metabolism

The major results may be summarized as follows: Chronic administration of excessive amounts of adrenocortical steroids alters electroshock seizure threshold (EST); deoxycorticosterone acetate (DCA) increases EST (decreases excitability), whereas cortisone acetate and cortisol acetate decrease it (increase excitability); corticosterone has little effect. 11-Deoxy-17-hydroxycorticosterone acetate elevates EST slightly, whereas 11-dehydrocorticosterone acetate decreases it slightly.

The increase in EST induced by a single dose of DCA was associated with a decrease in intracellular brain Na concentration, an increase in ratio of extracellular to intracellular brain Na, an increase in brain concentrations of glutamic and aspartic acids and a decrease in brain concentrations of glutamine and asparagine, in contrast, the decrease in EST induced by a single dose of cortisol was associated with the opposite effects On the basis of these facts, DCA and cortisol are postulated to affect, in opposite manner, the active transport of Na across brain cells and thereby to modify brain excitability The glutamic acid-glutamine and the aspartic acid-asparagine systems are thought to be involved in the process of Na transport

Aldosterone, the natural electrolyte-regulating hormone of the adrenal cortex, was found to have less effect on EST than did an equivalent dose of DCA

The duration of the postictal depression which follows maximal electroshock seizures in rats was found to be correlated with the blood sugar level, but unrelated to brain excitability or brain electrolyte metabolism

On the basis of results derived from a study of the effects of certain central nervous system drugs on the pituitary-adrenal system, the conclusion has been reached that the adrenal cortex has a regulatory influence on the excitability of the brain This regulatory function is operative only when changes in excitability occur, and the adrenocortical hormones then act so as to restore normal brain excitability, regardless of the direction of the original deviation However, this normal regulatory activity may be modified if the drug being tested activates the pituitary-adrenal system and thereby changes the normal pattern of adrenocortical secretion. Examples of such drugs' effects are presented, and it is suggested that these drugs stimulate the pituitary-adrenal system by virtue of an action directly on the central nervous system.

## ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. R. E. Knauff, Upjohn Co., for the gift of aldosterone. They also acknowledge the valuable technical assistance of Miss Adelia Baird during the course of these experiments. The advice of Dr. Louis S. Goodman throughout the course of this investigation has been of inestimable value and is greatly appreciated by the authors.

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DISCUSSION

CHAIRMAN H. HOAGLAND: This excellent paper is now open for discussion. I would like to ask you, Dr. Woodbury, if you regard the changes in brain potassium following adrenalectomy and replacement therapy entirely secondary and compensatory to movements of sodium

D. M. WOODBURY: This is true under most conditions although we have some evidence from other studies that potassium, like sodium, is actively transported in the brain, but in the case of these steroids both DOCA and cortisone produce identical effects on potassium ratio across the brain whereas they produce opposite effects on sodium ratio

G. PINCUS: Did you use corticosterone in combination with these to get that effect?

D. M. WOODBURY: I did. Corticosterone given acutely will antagonize the effects of both DOCA and cortisone

F. ELMADJIAN: Would this be true for ACTH?

D. M. WOODBURY: Yes

B. B. BRODIE: How do you know whether the sodium is in the extra- or in the intracellular space?

D. M. WOODBURY: We have used the chloride space to measure the extracellular fluid volume in the brain. We have some good evidence, which I don't have time to present, which shows that the chloride in brain is in rapid equilibrium with the chloride in plasma. In addition, some data we have accumulated on the uptake of radioactive chloride and sulfate by brain of rats demonstrates that the chloride space provides a good estimate of extracellular fluid volume in the brain.

B. B. BRODIE: How would you know that these hormones do not change the permeability to chloride?

D. M. WOODBURY: This is certainly one possibility and, as a matter of fact, on chronic administration, cortisone increases the chloride space in brain and this complicates the matter of calculating intracellular sodium concentration. On acute administration of cortisone, however, there is no change in the chloride space. In contrast, chronic treatment with DOCA does not alter chloride space. This effect of chronic cortisone treatment on chloride space may be related to the known effects of this steroid on glial tissue of brain or to the shift of hydrogen ions across cell membranes which occurs during cortisone administration as discussed in the text

C. H. GLASER: I would like to make some comments concerning the clinical counterparts of this work which Dr Woodbury so beautifully presented. For example, cortisone has occasionally produced spontaneous seizures in patients who otherwise have been not predisposed to seizures. These have been patients with rheumatoid arthritis, asthma, and other illnesses

In studying epileptic patients in clinical experimentation, we have found that the administration of cortisone and ACTH over two or three weeks will worsen the electroencephalograms in some instances and produce spike-wave

discharges which had not existed previously. These epileptic patients, however, did not have any specific increase in their seizures, just increased EEG abnormality. Along with this it has been demonstrated that occasionaly the electroencephalograms of neurologically normal subjects have been made abnormal by the administration of these hormones We have found that the administration of a single dose of hydrocortisone intravenously over a 4-hour period would worsen the electroencephalogram of epileptics and bring on seizure discharges which were not present previously We did not precipitate clinical seizures, however. These observations certainly run parallel to those of Dr. Woodbury and his group

The Dilantin effect is certaintly interesting and here again we notice certain clinical counterparts. One of the mysteries of the effect of Dilantin has always been that in order to observe its effect as an anticonvulsant one always had to wait about 24–48 hours after the initial administration. In acute situations other anticonvulsants have to be used Since the anticonvulsant effect of Dilantin does not take effect before this time it is very possible that the initial stimulation effect on the adrenal cortex which would lower the cerebral excitability or not change it very much, as shown by Dr Woodbury, is related to the increased hydrocortisone output We did some preliminary experiments on human epileptics and studied their urinary output of adrenal steroids We measured only the 17-ketosteroids and oxysteroids and here acute administration of Dilantin initially raised the urinary output of these products, but after 2 to 8 weeks, say a week or 2 or 3 weeks, the output of these products was decreased We thought originally that this meant an eventual depressant effect on the adrenal system. However, I now realize that we probably were not measuring Compound B or its related output We did not measure the plasma concentration and it is probably the differential effect on the hormones that allows Dilantin to raise the threshold after a certain period of time This work of Dr Woodbury is a very nice example of applied pharmacology These investigations that have been carried out in the laboratory may help us in explaining some of the confusing matters that we have been working with clinically.

I H Page· Would you say that is a complete explanation?

G H Glaser No

I. H. Page How important is the adrenal cortex? Let us put it this way, that the clinical effect seems so minor, from the nice effects you get in the animals in changes in threshold, does that mean you just don't give enough or does it mean that this just isn't the whole story?

G. H Glaser Clinical effects of what, the hormone?

I H Page Hormones the changes, for instance, in vascular reactivity which are mediated in part at least by the central nervous system don't seem to be very much affected by the administration of adrenal corticoids

G. H. Glaser We, of course, wondered about this It is certainly only a partial effect. I do not think it is the whole story at all One way to look at it is that this system is one way in which the electrolytes are controlled or regulated and there may be other ways

I H Page. Is this the adrenalectomized patient you are talking about? The totally adrenalectomized patient seems to be to all intents and purposes clinically almost normal, provided he is receiving adequate therapy I have always been

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by the administration of 4–15 mg. of deoxycorticosterone acetate daily as sublingual tablets (*J. Am Med Assoc* **145**, 715, 1951) I am impressed, like Dr Glaser, with the similarity between the clinical results and Dr Woodbury's data on the rat's electroshock threshold The only discordant agent is corticotropin which is quite convulsant in man, especially in children, and not so in the rat Dr. Woodbury has explained that now by showing that the principal product invoked by corticotropin in the rat is corticosterone which is not so convulsant as cortisol, which is the principal steroid elaborated in man

I have one question Since the sodium effect seems to be so important, what about the convulsant action of the Δ-1 compounds which are far less potent, at least as far as total body sodium and potassium are concerned? I don't know about their effect upon brain electrolytes Perhaps you do.

D. M. WOODBURY· I have not tried these compounds as yet, but intend to do so as soon as possible

G PINCUS· I would like to ask two questions According to Farrell, 11-deoxycorticosterone is found in adrenal vein blood A question I would like to ask, is it enough? He finds it in the dog

D M WOODBURY· According to G L Farrell *et al* (*Proc Soc Exptl Biol Med* **87**, 587, 1954) the small amount of DOC which is secreted by the adrenal gland of the dog is probably not sufficient to make a significant contribution to the metabolic activities of the adrenal cortex under normal conditions, but it is possible that under certain altered conditions its secretion is increased and it might play a role in these situations

I would also like to make a point Dr Page mentioned the lack of effects of excessive amounts of these steroids on the vascular system I think we have to consider two types of roles of the adrenal steroids. One is an active participation of the steroid itself, as for example, its effects on the nervous system The other is the so-called permissive effect as described by Ingle, it is particularly well seen in the cardiovascular system If no adrenocortical hormone is present the blood vessels lose their sensitivity to such agents as epinephrine and acetylcholine, whereas if an optimal amount of steroid is present normal sensitivity to these agents is restored If excessive quantities of steroid are present, however, no increased sensitivity to the agents results The excitability of the nervous system, on the other hand, is sensitive to the actual level of steroid in the blood, increasing the level increases its effect on the nervous system

G. H GLASER There have been some measurements, by the Kety-Schmidt method, of cerebral blood flow during cortisone administration It is my recollection that the change in cerebral flow was only about minus 2% However, the cerebral vascular resistance increased in the order of about 12 to 15%, which may not be significant, but at least this is more of a change than that in cerebral blood flow The significance of these findings is not very clear, but there is this indication that there is no significant effect on blood flow itself by the hormones

A G SLOCOMBE. In measuring the excitability of whatever part of the cortex gives rise to the so-called dendritic potentials, I found no significant difference between adrenalectomized and normal rats This suggests to me that the site of the excitability found by you is different, and the site of the effect of adrenalectomy on α-frequencies may be at discrete steroid-sensitive areas of the brain I wonder if you would like to speculate on which areas these might be Concepts of this

surprised at how normal, not how abnormal, he is I thought when we first did the operation we were doing something terrible, but actually it was surprising how little adverse effect there was.

G. PINCUS: I would like to point out to Dr. Page that the adrenalectomized human being is not a very happy object. The only way the person is kept alive is to have medication and careful management, particularly in stressful situations.

CHAIRMAN H. HOAGLAND: In rats that are bilaterally adrenalectomized there are definite changes in aspects of brain function, quite aside from the threshold effects that Dr. Woodbury has talked about. There is a decline in the frequency of the electroencephalogram. One also sees a decline in frequency in acute Addisonian patients, or in adrenalectomized patients temporarily taken off replacement therapy Dr Bergen and I showed several years ago that the decline in the frequency of the electroencephalogram parallels a significant decrease in oxygen consumption of the brain in the adrenalectomized rat and also that this is correlated with a decrease in cerebral circulation. We also found evidence that the decline in cerebral circulation is due to a decrease in blood pressure and a decrease in the general tone of the systemic circulation and probably is not due to the direct effects on the brain circulation *per se*. Correlated with these effects there were also changes in velocity of conduction from foot to cerebral cortex of electrical response, but here again these were extreme effects of total adrenalectomy. Normal effects could be restored by cortisone and by adrenocortical extracts and by certain other steroids, but not by DCA

S. UDENFRIEND I wonder, since you are talking about stress conditions, whether the medullary part might be considered to play a role, and whether adrenal epinephrine or norepinephrine may be involved

CHAIRMAN H. HOAGLAND. The medulla may very well play a role We were, however, able to restore the depressed functions by adrenal cortical extract and by cortisone without the administration of either epinephrine or norepinephrine There is evidence of a synergistic action in restoring circulation between norepinephrine and adrenocorticoids as shown by Rachmael Levine and his collaborators, but we were able to restore quite fully the EEG patterns, conduction velocity to the cortex and oxygen consumption with steroids alone

H J KOCH, JR I would like to ask Dr Woodbury, if he feels that the normalizing of electric shock threshold is a function of electrolyte shift such as he measured, or whether he thinks this is a parallel phenomenon

D M WOODBURY I think they are the function of the electrolyte shifts, but we do not have sufficient data as yet to prove this

B B BRODIE Does the administration of any of the adrenal steroids influence epileptic seizures?

D M WOODBURY DOCA has already been used in the treatment of epilepsy.

B. B BRODIE How useful is it?

D. M. WOODBURY I would like Dr Gordan to answer that question

G. S GORDAN This was originally studied by *I McQuarrie, M R Ziegler*, and *J A Anderson* in Minneapolis (*Endocrinology* 2, 408, 1942) They reported two cases of refractory epilepsy relieved by administration of DCA R B Aird's two cases were not (*J Nervous Mental Disease* 99, 501, 1944) Subsequently we tried it by the double-blind technique in a group of ten epileptic patients who were refractory to all previous medication The frequency of attacks was reduced significantly

# *In Vitro* Effects of a Steroid Anesthetic On Brain Metabolism

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The anesthetic properties of certain steroid hormones were first reported by Selye in 1941 (23). He then systematically investigated 75 steroids in this regard (24) and concluded that steroids oxygenated only at opposite ends of the molecule and containing no double bonds in the rings were the most potent anesthetics He also found no correlation between hormonal and anesthetic activity.

Recently, pregnane-3,20-dione-21-ol sodium hemisuccinate (sodium hydroxydione) has been described as a potent, soluble anesthetic without serious side effects and devoid of hormonal or salt-retaining properties except in enormous doses (12, 19). Clinical trials (8, 18) have indicated that in man it produces loss of consciousness accompanied by moderate muscular relaxation, minimal respiratory depression, and some degree of analgesia. The principal disadvantages are a tendency to cause thrombophlebitis unless injected in a concentration of 1% or less and insufficient analgetic activity. It appears to be most useful as a basal anesthetic supplemented with nitrous oxide, analgetics, and relaxants as required

Its actions on cerebral metabolism *in vivo* have been studied in man (4) and it has been found to decrease cerebral blood flow, oxygen uptake, and glucose uptake to the same degree as barbiturate-meperidine anesthesia.

The actions of sodium hydroxydione on cerebral metabolism *in vitro* have not been studied extensively although Quastel reported (21) that it inhibited glucose oxidation in brain cortex preparations and pyruvate oxidation in brain mitochondria. It has been shown that the anesthetic potency of certain steroids is correlated with their ability to inhibit the oxygen uptake of rat brain homogenates (3). Furthermore, they inhibited oxidative reactions at the dehydrogenase level rather than at the flavoprotein-cytochrome level as is the case for other anesthetics (3, 6, 7). This difference in action plus the introduction of sodium hydroxydione

nature are becoming increasingly important as people are finding such sites for other compounds

D. M. WOODBURY: I don't think we can answer that question until we do experiments on the effects of cortisone in animals which have been subjected to ablation at different levels of the nervous system or by testing the effects of the adrenocortical steroids on discrete areas of the brain by use of stimulating and recording techniques However, the work of G A. Winter and L. Flataker (*J. Pharmacol Exptl. Therap.* 103, 93, 1951) suggested that cortisone acts on the entire cerebrospinal axis.

In this connection, the work of G. Misrahy and J. E P. Toman (*Federation Proc* 12, 352, 1953) is of interest They measured the effects of drugs on irradiation of cerebral cortical electrical responses in the rabbit The phenomenon of "irradiation" or the process reflected in a spread of the response from stimulated areas of cerebral cortex to distant areas was quantitated and the effects of cortisone were tested Cortisone reduced the ratio of contralateral to ipsilateral threshold for all responses, which is an excitant effect of this steroid This type of study should be continued

R W. GERARD: There is a small region around the mammillary body, the stimulation of which can greatly increase the oxygen consumption of the cerebrum, and this may perhaps have been involved in the changes in metabolism that can be induced by adrenomedullary hormone administration I don't know whether one can guess about the cortical ones

May I ask two questions that seem to me to deserve some attention One, I noticed that your slides in many cases indicated the variance of the results, in some cases did not Looking at the heights of the bars in the cases where the variance was indicated, in many cases the differences were negligible as compared to the variance Did you personally feel that the differences were significant?

D M WOODBURY The differences between means were significant in all cases where they were mentioned

R W. GERARD Whenever you mentioned the phenomenon these differences were statistically significant?

D M WOODBURY Yes

R W. GERARD I am glad to have that brought out because I was not certain of it The other thing I would like to hear some discussion on, if I did not partly misunderstand, what you seem to have is a system in which whatever type of corticoid is necessary is released to bring the threshold back to normal from either above or below normal values How does the action of the brain, down through all these systems give the right kind of discharge?

D M WOODBURY Since the center for central regulation of aldosterone release seems to be separate from that for the regulation of release of ACTH and that for adrenal medullary hormones, it seems to me that release of these various hormones as a result of the elevation or lowering of threshold caused by the administered agent, and the resulting "normalizing" effect can be explained by an action of the substance which influences the excitability of the brain on one of these three centers of regulation of hormone release However, much further research is necessary to reveal the finer control mechanisms

R. W. GERARD· I wonder with the specific receptor mechanism—since aldosterone is one type and ACTH is another type—if you can get a distinction

D. M. WOODBURY: Yes, a distinction can be obtained by these mechanisms

Sodium hydroxydione and sodium pentobarbital were soluble and could be added in solution, but when insoluble steroids were used they were ground to a fine powder in an agate mortar, suspended in the appropriate medium, and added as a suspension

Some studies utilized the technique of Huston and Martin (10) for determining the rate of respiration of brain slices in contact with oxygen. Slices were spread out on fiber-glass mats which permitted practically free diffusion of oxygen into the side of the slice in contact with the mat. These preparations were then placed in wide-mouthed Warburg vessels for measurement of oxygen uptake. One ml. of glucose-Ringer was present in a large side arm to maintain a moist atmosphere in the vessel. This could be tipped into the main compartment of the vessel when so desired

*Glucose uptake and glycolysis studies* Glucose was determined by the anthrone method (1) Lactic acid was determined by a modified method of Miller-Muntz (17) Typically, two or three vessels were removed from the bath just after the initial reading of the manometers and the suspending medium analyzed for glucose and/or lactic acid. At

and the influence of the steroid evaluated

## II. RESULTS

It is now generally accepted that in concentrations effective *in vivo* anesthetics do not inhibit the $Qo_2$ of brain slices suspended in conventional media However, when slices are stimulated electrically (13) or are permitted to respire in media containing a high concentration of potassium or certain concentrations of dinitrophenol (2, 15) the $Qo_2$ is increased and the extra oxygen uptake is sensitive to pharmacologically active concentrations of anesthetics

For these studies, a high potassium medium was selected to produce the elevated respiratory rate and sodium hydroxydione was compared with sodium pentobarbital in regard to its ability to depress the $Qo_2$ of brain slices respiring in extracellular glucose-Ringer's solution (EGR) without KCl, or with 0 1 *M* KCl (KCl EGR). Figure 1 shows that in KCl EGR the $Qo_2$ was elevated, but progressively declined after the first 30 minutes of a 2-hour run. At 30 minutes the stimulation in KCl EGR was 66%, by 120 minutes it had fallen to 14%. Sodium pentobarbital ($2 \times 10^{-4}$ *M*) did not appreciably inhibit oxygen uptake in EGR but immediately depressed

as a useful anesthetic prompted this study on the effects of this agent on cerebral metabolism *in vitro*. Although we realize that inhibition of oxidative reactions may not fully explain the phenomenon of anesthesia (9) we feel that a comparison of the actions of different classes of anesthetics both *in vitro* and *in vivo* may contribute to the elucidation of their mechanism of action.

## I. METHODS

*Source of tissue.* The animals used in these studies were adult male Sprague Dawley rats and adult guinea pigs of both sexes. They were decapitated and slices or homogenates were prepared from the prosencephalon. Cerebral cortex slices approximately 0.5 mm. thick were cut freehand with a razor and template. Slicing was done in a refrigerated box the atmosphere of which was kept moist by a spray of water from a nebulizer. A torsion balance was used for weighing out approximately 40-mg. samples of slices which were immediately placed in previously prepared Warburg vessels. For some experiments the prosencephalon was weighed, homogenized for 30 seconds in a loose-fitting Potter instrument and diluted to give a 10% suspension of tissue which was pipetted into the Warburg vessels.

Human brain tissue from frontal lobotomy operations or in one case from an occipital lobe was obtained during surgery and carried to the laboratory in an ice cold beaker. After removal of the adhering pia mater, cerebral cortex slices were prepared and handled in the usual manner.

*Oxygen uptake studies* The respiration of slices or homogenates was determined at 37.3° C by means of conventional Warburg techniques. Carbon dioxide was absorbed in 0.1 ml. of 10% KOH. The gas phase was oxygen for slices and air for homogenates. The suspending medium contained 0.145 *M* NaCl, 0.004 *M* KCl, 0.0016 *M* $CaCl_2$, 0.0011 *M* $MgSO_4$, 0.01 *M* $Na_2HPO_4$ buffer pH 7.35, and various organic substrates. The usual substrate concentrations were as follows: glucose 0.01 *M*, sodium pyruvate 0.02 *M*, sodium glutamate 0.017 *M*, and sodium succinate 0.009 *M*. A 0.1 *M* potassium medium was prepared by adding 7.45 mg. KCl per milliliter to any of the above media.

Oxygen uptake was usually measured for 2 hours following a 10-minute equilibration period in the 37.3° C bath. Manometer readings were made every 15 minutes and $Q_{O_2}$'s were calculated on a wet weight basis. Steroids or sodium pentobarbital were added from the sidearms of the Warburg vessels immediately after the initial manometer readings

as a substrate it seemed desirable to investigate its effects using other substrates. Preliminary runs indicated that pyruvate and succinate oxidations were only slightly, if at all, affected by 2.5 × $10^{-4}$ $M$ sodium hydroxydione so for subsequent studies the concentration used was increased to 5 × $10^{-4}$ $M$. Figure 2 shows the effects of this concentration on the respiration of rat cerebral cortex slices in EGR and KCl EGR, in pyruvate-Ringer's (EPR) and KCl EPR and in succinate-Ringer's (ESR).[1]

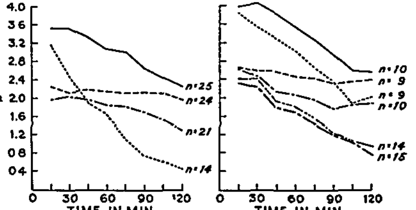


FIG 2 The effects of sodium hydroxydione on the oxygen uptake of rat cerebral cortex slices in various substrates with and without 0 1 $M$ KCl

| *Graph on left* | *Graph on right* |
|---|---|
| – – – EGR control | – – – EPR control |
| ——— KCl EGR control | ——— KCl EPR control |
| –—– EGR + 5 × $10^{-4}$ $M$ Na hydroxydione | –—– EPR + 5 × $10^{-4}$ $M$ Na hydroxydione |
| ····· KCl EGR + 5 × $10^{-4}$ $M$ Na hydroxydione | ····· KCl EPR + 5 × $10^{-4}$ $M$ Na hydroxydione |
| $n$ indicates the number of vessels | ‐‐‐‐ ESR control |
| | -—— ESR + 5 × $10^{-4}$ $M$ Na hydroxydione |
| | $n$ indicates the number of vessels |

For EGR and KCl EGR the inhibition was slightly greater than that produced by the lower concentration, in EGR it was insignificant at 30 minutes but was 34% at 120 minutes In KCl EGR inhibition amounted to 30% at 30 minutes and 79% at 120 minutes. Development of inhibition was delayed in EGR but was prompt in KCl EGR The same results were obtained with guinea pig cerebral cortex slices.

[1] The letter E in these abbreviations means extracellular.

the excess oxygen uptake produced in KCl EGR. At 30 minutes inhibition was 27%; at 120 minutes it was 18%. Inhibition in KCl EGR did not exceed that produced in EGR as reported by Ghosh and Quastel for other agents (2). In contrast, sodium hydroxydione ($2.5 \times 10^{-4}\ M$) in a concentration which slightly inhibited oxygen uptake of brain slices respiring in EGR produced a slightly delayed but progressively increasing inhibition in KCl EGR. At 30 minutes inhibition was only 16%; by

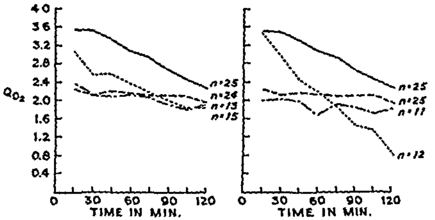


FIG. 1. A comparison of the effects of sodium pentobarbital and sodium hydroxydione on the oxygen uptake of rat cerebral cortex slices in glucose-Ringer's and glucose-Ringer's plus 0.1 $M$ KCl

*Graph on left*

- – – – EGR control
- ——— KCl EGR control
- –·–·– EGR + $2 \times 10^{-4}\ M$ Na pentobarbital
- ······ KCl EGR + $2 \times 10^{-4}\ M$ Na pentobarbital
- n indicates the number of vessels

*Graph on right*

- – – – EGR control
- ——— KCl EGR control
- –·–·– EGR + $2.5 \times 10^{-4}\ M$ Na hydroxydione
- ······ KCl EGR + $2.5 \times 10^{-4}\ M$ Na hydroxydione
- n indicates the number of vessels

120 minutes it was 62%. The succinate moiety of the molecule, if available as substrate, could have no more than a minimal effect on these values since $5 \times 10^{-4}\ M$ sodium succinate elevated the $Q_{O_2}$ only about 10% above the endogenous level for the first 60 minutes. Thus, unlike other agents studied (2), this steroid anesthetic inhibited oxygen uptake in the presence of 0.1 $M$ KCl more than it inhibited it in conventional substrates.

Since sodium hydroxydione could inhibit oxygen uptake with glucose

as a substrate it seemed desirable to investigate its effects using other substrates. Preliminary runs indicated that pyruvate and succinate oxidations were only slightly, if at all, affected by $2.5 \times 10^{-4}$ *M* sodium hydroxydione so for subsequent studies the concentration used was increased to $5 \times 10^{-4}$ *M*. Figure 2 shows the effects of this concentration on the respiration of rat cerebral cortex slices in EGR and KCl EGR, in pyruvate-Ringer's (EPR) and KCl EPR and in succinate-Ringer's (ESR).[1]

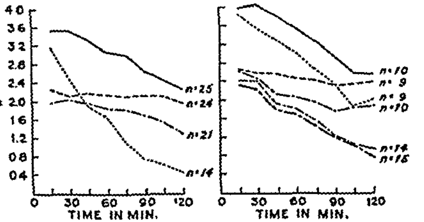


FIG. 2. The effects of sodium hydroxydione on the oxygen uptake of rat cerebral cortex slices in various substrates with and without 0.1 *M* KCl

| *Graph on left* | *Graph on right* |
|---|---|
| – – – EGR control | – – – EPR control |
| ——— KCl EGR control | ——— KCl EPR control |
| –·–·– EGR + $5 \times 10^{-4}$ *M* Na hydroxydione | –·–·– EPR + $5 \times 10^{-4}$ *M* Na hydroxydione |
| ····· KCl EGR + $5 \times 10^{-4}$ *M* Na hydroxydione | ····· KCl EPR + $5 \times 10^{-4}$ *M* Na hydroxydione |
| n indicates the number of vessels | - - - - ESR control |
| | ·——— ESR + $5 \times 10^{-4}$ *M* Na hydroxydione |
| | n indicates the number of vessels |

For EGR and KCl EGR the inhibition was slightly greater than that produced by the lower concentration, in EGR it was insignificant at 30 minutes but was 34% at 120 minutes. In KCl EGR inhibition amounted to 30% at 30 minutes and 79% at 120 minutes. Development of inhibition was delayed in EGR but was prompt in KCl EGR. The same results were obtained with guinea pig cerebral cortex slices.

[1] The letter E in these abbreviations means extracellular

Other substrates, however, presented a different picture. Ghosh and Quastel (2) found that when the substrate was pyruvate the anesthetics they used prevented the stimulation produced by 0.1 *M* KCl. Sodium hydroxydione produced only minimal inhibition in KCl EPR at a concentration which produced a significant and slightly delayed inhibition in EPR. At 30 minutes inhibition in EPR was insignificant; at 120 minutes it was 21%. In KCl EPR inhibition appeared promptly (12% at 30 minutes) and increased only slightly during the remainder of the run (21% at 120 minutes). Thus, sodium hydroxydione only slightly inhibited the potassium stimulation when pyruvate was the substrate. There was no increased inhibition when the concentration of pyruvate was lowered to $2 \times 10^{3}$ *M* or $5 \times 10^{4}$ *M*; hence in this preparation there is apparently no competition for the pyruvate oxidase system as reported by Persky *et al* (20) for sodium pentobarbital.

With sodium succinate as substrate (ESR), sodium hydroxydione produced essentially no inhibition. KCl ESR was not used since it has been shown that for rat brain slices potassium does not stimulate oxygen uptake in the presence of succinate (2).

Rat brain slices have a very low endogenous respiration and oxygen uptake is not raised above the endogenous level by sodium glutamate. Consequently, guinea pig cerebral cortex slices were used to investigate the effects of sodium hydroxydione on the oxygen uptake of brain slices respiring in Ringer's containing no added substrate (ER) or with sodium glutamate as substrate (EGlut) Figure 3 presents the results under these conditions. The endogenous respiration was appreciable although not well maintained. At 120 minutes the $Qo_2$ was 49% of the value at 15 minutes. The addition of 0.1 *M* KCl produced a stimulation which lasted less than 30 minutes after which the $Qo_2$ fell below the level in ER. Presumably stimulation was maintained only until endogenous substrates were exhausted Sodium hydroxydione slightly inhibited oxygen uptake in both ER and KCl ER. In ER inhibition was delayed for 45 minutes; in KCl ER it was immediate and most prominent during the first hour presumably while endogenous substrates were being utilized.

Sodium glutamate (EGlut) maintained the oxygen uptake of guinea pig cerebral cortex slices well above the endogenous level and potassium produced a stimulation which was not as marked as with the other substrates. At 30 minutes the $Qo_2$ was 54% higher in KCl EGlut than in [illegible] Sodium hydroxy- [illegible] and KCl EGlut, the pattern resembling that produced when the substrate was pyruvate.

In EGlut inhibition at 30 minutes was 19%; by 120 minutes it had increased to 28%. In KCl EGlut the $Q_{O_2}$ was depressed 26% at 30 minutes and 35% at 120 minutes

Methylene blue, $6 \times 10^{-5}$ *M*, was used to determine the sensitivity to sodium hydroxydione of the dehydrogenases involved in glucose oxidation. Since the methylene blue reversal of inhibition produced by anesthetics is not apparent with slices, the study was done with brain

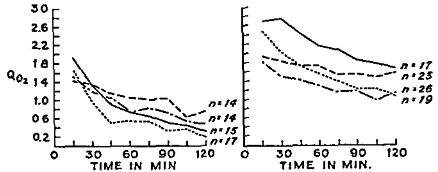


FIG 3 The effects of sodium hydroxydione on the oxygen uptake of guinea pig cerebral cortex slices in the absence of added substrate and in sodium glutamate with and without 0 1 *M* KCl

| *Graph on left* | *Graph on right* |
|---|---|
| – – – ER control | – – – EGlut control |
| ——— KCl ER control | ——— KCl EGlut control |
| –·—– ER + $5 \times 10^{-4}$ *M* Na hydroxydione | –·—– EGlut + $5 \times 10^{-4}$ *M* Na hydroxydione |
| ----- KCl ER + $5 \times 10^{-4}$ *M* Na hydroxydione | ----- KCl EGlut + $5 \times 10^{-4}$ *M* Na hydroxydione |
| *n* indicates the number of vessels | *n* indicates the number of vessels |

homogenates. Figure 4 shows that the inhibition of oxygen uptake in EGR produced by $5 \times 10^{-4}$ *M* sodium hydroxydione is reversed by methylene blue. The same result was obtained when the concentration of sodium hydroxydione was raised to $1 \times 10^{-3}$ *M*. Since the $Q_{O_2}$ values obtained with the steroid plus methylene blue were not quite as high as those obtained with methylene blue alone, sodium hydroxydione may have some inhibitory action on dehydrogenases but at least they are relatively insensitive to it. This finding is directly opposite to that reported for other steroids (3) which, by this technique, were found capable of inhibiting dehydrogenases

Findings to this point have been obtained with the use of agents added *in vitro* to brain preparations from untreated animals. The technique of Huston and Martin (10) for determining oxygen uptake of tissues in the absence of liquid suspending media and comparable methods reported by Rodnight and McIlwain (22) provide an approach to the study of *in vitro* effects of agents administered to animals *in vivo*. Huston and Martin (11) have shown that tissues from rats previously treated with

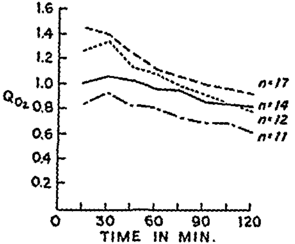


FIG 4. The effect of methylene blue on the inhibition of oxygen uptake of rat brain homogenates produced by sodium hydroxydione.

——— EGR control
– – – EGR + 6 × $10^{-5}$ M methylene blue
–·–·– EGR + 5 × $10^{-4}$ M Na hydroxydione
······ EGR + 5 × $10^{-4}$ M Na hydroxydione + 6 × $10^{-5}$ M methylene blue
n indicates the number of vessels

the metabolic stimulant dinitrophenol respired at higher rates on fiber-glass mats than they did in liquid media. Conversely, tissues from rats given sodium arsensite respired at lower rates on mats than in liquid media.

This technique was used to investigate the effects of sodium hydroxydione and sodium pentobarbital *in vivo* on the oxygen uptake of rat cerebral cortex slices. Rats were given either 250 mg per kilogram of

sodium hydroxydione or 50 mg. per kilogram of sodium pentobarbital intraperitoneally and decapitated 30 minutes later. These doses produced profound anesthesia with adequate ventilation as evidenced by the absence of cyanosis and respiratory rates of 50–94 per minute just before sacrifice. In KCl EGR the oxygen uptake of slices from rats treated with sodium pentobarbital was identical with that obtained for slices from untreated rats and $2 \times 10^{-4} M$ sodium pentobarbital added to the media at the initial manometer reading produced the same amount of inhibition in both cases. Essentially the same findings were obtained with slices from rats given sodium hydroxydione although $Q_{O_2}$'s were *slightly higher from the treated animals when no steroid* ($5 \times 10^{-4} M$) was added *in vitro*.

Oxygen uptake studies made with brain slices on fiber-glass mats are summarized in Table I

TABLE I[a]

| | $Q_{O_2}$ in contact with oxygen | | | $Q_{O_2}$ after addition of EGR at 60 minutes |
|---|---|---|---|---|
| Time in minutes | 15 | 30 | 60 | 90 |
| Control (22) | 3.0 | 2.0 | 1.1 | 1.4 |
| Na hydroxydione treated (17) | 3.5 | 2.1 | 1.2 | 1.5 |
| Na pentobarbital treated (17) | 3.1 | 2.1 | 0.6 | 1.5 |

[a] Figures in parentheses indicate number of vessels

Initial $Q_{O_2}$ values for slices from control animals were higher than obtained in conventional media but not as high as in KCl EGR or KCl EPR The respiratory rate fell off rapidly with some recovery after adding EGR at 60 minutes. Since recovery was not complete, it must be assumed that some degeneration of oxidative processes occurred before the EGR was added.

Slices from sodium hydroxydione-treated rats respired at a significantly higher rate initially than slices from control animals ($P < 0.01$ at 15 minutes) but after the first 15 minutes respired at the same rate as controls. In contrast, slices from sodium pentobarbital-treated rats had practically the same initial $Q_{O_2}$ as controls but at 60 minutes the respiratory rate was significantly lower ($P < 0.01$) than found for either of the other groups After adding EGR, slices from all groups respired at the same rate While these results are not yet explained, they indicate

a difference in action between sodium hydroxydione and sodium pentobarbital and emphasize the potential value of the mat technique.

The delay in onset of anesthesia manifested after the intravenous administration of sodium hydroxydione (18, 19) suggested that the compound might be metabolized to a more active compound. Two known metabolites, pregnane 3(α) and pregnane 3(β)-21-diol-20-one were made available to us and compared with pregnane-3,20-dione-21-ol (hydroxydione) as to their ability to inhibit the oxygen uptake of rat brain homogenates in EGR. As shown in Fig. 5, neither metabolite was as

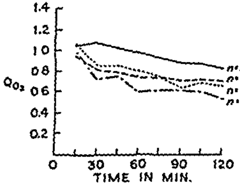


FIG. 5. A comparison of the effects of sodium hydroxydione and two of its metabolites on the oxygen uptake of rat brain homogenates

—— EGR control
- - - - - pregnane 3(β)21,diol-20-one
— — — pregnane 3(α)21,diol-20-one
— - — - pregnane 3,20,dione-21-ol (P66)
} 7.5 × $10^{-4}$ M
*n* indicates the number of vessels

potent as the parent compound. In addition, another related compound, allo-deoxycorticosterone acetate, was even less potent as an inhibitor.

Preliminary studies on the influence of sodium hydroxydione on aerobic glycolysis and glucose uptake are presented in Table II.

The data show that in EGR, sodium hydroxydione produced approximately 100% stimulation of aerobic glycolysis with both rat and guinea pig brain slices. Similar marked stimulation of lactic acid production has been reported for other anesthetics (9). As might be expected, glucose uptake was also increased in both EGR and KCl EGR. A few experiments using human brain slices provided similar results. Concentrations of sodium hydroxydione from 5 × $10^{-4}$ M to 6 × $10^{-3}$ M stimulated both aerobic glycolysis and glucose uptake in EGR and KCl EGR but

TABLE II

THE EFFECT OF 5 × $10^{-4}$ *M* SODIUM HYDROXYDIONE ON AEROBIC GLYCOLYSIS AND GLUCOSE UPTAKE[a]

| Species | Substrate | Zero time | 2 hr. control | 2 hr. Na hydroxydione |
|---|---|---|---|---|
| | | γ lactic acid/mg. tissue | | |
| Rat | EGR | $1.4^{(1.3-1.6)}_{(4)}$ | $2.9^{(2.4-3.4)}_{(5)}$ | $6.4^{(5.7-7.9)}_{(6)}$ |
| Guinea pig | EGR | $1.4^{(1.1-1.9)}_{(4)}$ | $4.2^{(3.6-5.8)}_{(4)}$ | $8.4^{(6.0-11.1)}_{(4)}$ |
| Rat | KCl EPR | $1.4^{(1.0-2.0)}_{(4)}$ | $3.6^{(1.5-5.2)}_{(6)}$ | $2.9^{(1.1-4.6)}_{(7)}$ |
| Guinea pig | EGlut | $1.1^{(0.6-1.9)}_{(3)}$ | $0.4^{(0.0-1.0)}_{(4)}$ | $0.3^{(0.1-0.4)}_{(4)}$ |
| | | γ glucose taken up/mg. tissue | | |
| Rat | EGR | $4.8^{(4.5-5.0)}_{(2)}$ | $20.7^{(17.0-22.4)}_{(3)}$ | $29.0^{(20.4-35.2)}_{(4)}$ |
| Rat | KCl EGR | $0.0_{(2)}$ | $15.6^{(14.7-17.6)}_{(3)}$ | $27.6^{(23.7-30.9)}_{(4)}$ |

[a] Figures in parentheses indicate number of vessels (subscript) and range (superscript).

$1 \times 10^{-4} M$ had no effect except that stimulation was noted in one run using a combined glucose-glutamate substrate.

Lactic acid production was also studied with two other substrates. Table II shows that with guinea pig brain slices in EGlut sodium hydroxydione had little if any effect on it. Thus, the steroid does not appear to interfere with utilization of endogenous lactic acid nor does it cause the production of lactic acid with glutamic acid. With rat brain slices in KCl EPR plus sodium hydroxydione, lactic acid accumulation was not as great as in control vessels

## III. DISCUSSION

Although much remains to be done, some actions of sodium hydroxydione on brain metabolism have been elucidated by work reported above and certain comparisons can be drawn between its actions and those of other anesthetics.

As is the case with other agents, concentrations which produce anesthesia *in vivo* had little effect on oxygen uptake in the usual media but definitely inhibited the stimulated $Q_{O_2}$ produced by potassium ions. The concentrations which produced the findings illustrated in Fig 1 corresponded to 50 mg. per kilogram of sodium pentobarbital and 115 mg per kilogram of sodium hydroxydione This dose of sodium pentobarbital injected intraperitoneally produced profound anesthesia with adequate ventilation in 6 rats which were sacrificed at 30 minutes and the same effects were noted in 6 rats which received 250 mg. per kilogram of sodium hydroxydione under similar conditions. However, the data indicate that the actions of sodium hydroxydione on the stimulated $Q_{O_2}$ differ from those of sodium pentobarbital. Sodium pentobarbital immediately inhibited the stimulated $Q_{O_2}$ and apparently inhibited only the excess oxygen consumption produced by potassium ions The inhibition by sodium hydroxydione was delayed at least 15 minutes and increased with time, finally markedly exceeding the inhibition in EGR. The delay in onset of inhibition, which was also found with other substrates, is reminiscent of the delay in onset of anesthesia noted with this agent (18, 19) The immediate onset of inhibition noted with homogenates (Fig. 4) suggests that this delay may be related to the permeability of the central nervous system to this agent The inhibition of more than the potassium stimulated $Q_{O_2}$ suggests that sodium hydroxydione has a more profound action on the systems responsible for glucose oxidation in high potassium media than do other anesthetics Furthermore, this steroid, like other anesthetics (9) increases glucose uptake and aerobic

glycolysis indicating that it blocks the entrance of glucose into the tricarboxylic acid cycle and not the glucolytic pathways.

Another notable difference between sodium hydroxydione and other anesthetics is that the steroid inhibited less in KCl EPR than in KCl EGR This, plus a similar pattern of inhibition in KCl EGlut provided supporting evidence that its major site of action is on glucose oxidation. However, the presence of a definite degree of inhibition in high potassium EPR and EGlut may indicate a second site of action, namely, on the oxidation of tricarboxylic acid cycle constituents

There is evidence that sodium hydroxydione may inhibit oxidative enzymes at the familiar point between the flavoproteins and cytochromes of the electron transfer chain (5, 16). Dehydrogenases, at least those involved in glucose oxidation, are relatively insensitive as shown by the reversal of inhibition in EGR by methylene blue Cytochromes are also unaffected since there was no inhibition when the substrate was sodium succinate. This is in marked contrast to the findings for other steroids (3) which inhibited at the dehydrogenase level.

The data obtained with guinea pig brain slices, in addition to showing that potassium-stimulated endogenous substrate oxidation is inhibited by sodium hydroxydione, indicated that these substrates were exhausted within 60 minutes since after that time inhibition was negligible This suggests a method for a comparative study of the endogenous respiration of various species.

The stimulation of glutamic acid oxidation by potassium may indicate a fundamental difference between potassium and electrical stimulation of brain slices since McIlwain (14) was unable to stimulate the $Qo_2$ of guinea pig brain slices when the substrate was glutamic acid. However, since the $Qo_2$ in KCl EGlut was not as high as that attained in KCl EGR or KCl EPR further study of the conditions of electrical stimulation might be fruitful

The glucose uptake and aerobic glycolysis studies are preliminary only but we have had sufficient experience with the methods to feel that we can draw certain conclusions from the small number of vessels used Thus, as with other anesthetics, increased aerobic glycolysis in EGR was shown in duplicate runs on brain slices from three species including man, and increased glucose uptake was shown for rat brain slices in both EGR and KCl EGR It is probable that additional analyses would show a greater glucose uptake in KCl EGR than in EGR, since this has been our experience in other work. The lactic acid analyses in EGlut show that sodium hydroxydione neither interferes with the utilization of endoge-

nous lactic acid nor produces a shunting of glutamic acid to lactic acid. It does, however, decrease the amount of lactic acid found in KCl EPR. This may indicate inhibition of pyruvic acid utilization with either decreased formation of lactic acid or preferential utilization of lactic acid in the presence of inhibited pyruvate metabolism. The findings with the relatively new glass mat technique (10) require additional studies before they can be interpreted properly. At this point they suggest a difference in action between sodium hydroxydione and sodium pentobarbital but sleeping times must be determined to insure that the *in vivo* doses used were comparable. The lack of effect of *in vivo* administration of anesthetics on the $Qo_2$ of slices respiring in liquid media plus the recovery of oxygen uptake rates to control levels after the addition of EGR to slices respiring on mats suggests that the technique is achieving its purpose in preventing the washing out of drugs administered *in vivo* as indicated by the findings of Huston and Martin (11) with dinitrophenol and sodium arsensite. Our studies clearly show that both anesthetics when given *in vivo* affect oxygen uptake as determined by this technique but neither inhibits it during the initial period when the $Qo_2$ is nearly as high as that produced by electrical, potassium, or dinitrophenol stimulation.

Finally, neither of the metabolites studied was as potent as hydroxydione in inhibiting the oxygen uptake of rat brain homogenates. This was not unexpected in view of Selye's generalizations regarding structure and anesthetic potency of steroids (24). Since the metabolites probably possess anesthetic activity this finding does not rule out the possibility that the activity of sodium hydroxydione is due to a metabolite (4) but neither does it support it.

In conclusion, it has been demonstrated that sodium hydroxydione has some of the actions on brain metabolism common to other anesthetics but differs significantly in other actions. Since the actions of sodium hydroxydione on cerebral blood flow, oxygen consumption, and glucose uptake *in vivo* are similar to those of other anesthetics (4) it is apparent that basic biochemical mechanisms cannot be deduced from such studies alone.

## IV. SUMMARY

Some actions of sodium hydroxydione on brain metabolism have been determined using manometric techniques and measurements of glucose uptake and/or lactic acid production.

Like other anesthetics, sodium hydroxydione in concentrations effective *in vivo* inhibited the potassium-stimulated $Qo_2$ of brain slices more than

it inhibited in the usual media However, unlike sodium pentobarbital, it inhibited more than the excess oxygen consumption produced by potassium when glucose was the substrate. Some inhibition of oxygen uptake was noted in high potassium media containing pyruvate, glutamate, or endogenous substrates but this was not as marked as when the substrate was glucose There was no inhibition when the substrate was succinate and methylene blue reversed the inhibition when the substrate was glucose These findings plus the fact that sodium hydroxydione stimulated aerobic glucolysis and glucose uptake suggest that the principal action of this agent on oxidative reactions is to inhibit the entrance of glucose into the tricarboxcylic acid cycle Inhibition of triboxcylic acid cycle substrates appears to be a secondary action and the site of action on the electron transfer chain appears to be the same as for other anesthetics

Experiments with brain slices respiring in contact with oxygen after *in vivo* administration of sodium hydroxydione and sodium pentobarbital indicated different but as yet unexplained actions of these agents on oxygen uptake as measured by this technique.

Two metabolites of hydroxydione were found to be less potent inhibitors of oxygen uptake than the parent compound


## ACKNOWLEDGMENT

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## Discussion

B. B. BRODIE. In interpreting your interesting findings, Dr. Elliott, it should be borne in mind that the steroid anesthetics are administered as carboxylic acid esters The steroid anesthetics have a pKa of about 4 and are probably almost completely ionized at physiological pH. Presumably, therefore, they are hydrolyzed enzymatically before they penetrate the blood-brain barrier I believe that this has been demonstrated experimentally Another point: I wonder if, knowing the effect of anesthetics on the enzyme systems you have studied, would you tell us anything as to their site of action in inducing anesthesia

H W ELLIOTT. I agree with you These studies only suggest sites of action on enzyme systems

B B BRODIE: Since the steroid anesthetics affected the enzymes, after stimulation, but not before, could this not be interpreted as meaning that their action is primarily on the excitatory process?

H W. ELLIOTT Exactly

B B BRODIE But isn't this like saying that a car does not go because something is wrong with the transmission, when actually something is wrong with the spark plugs?

H W. ELLIOTT: We would like to think that the *in vitro* techniques used to stimulate oxygen consumption may at least parallel what is going on *in vivo* This stimulated respiration is more sensitive to anesthetics than is respiration in conventional media.

B B BRODIE Or the anesthetics have no direct effect on the cellular enzyme systems, but depress the excitability of the cell as a result of which intracellular enzymes appear to be affected

H. W. ELLIOTT: I doubt it

G. S. GORDAN: The clinical importance of this work deserves clarification, 21-

hydroxypregnanedione-3,17 is not a perfect compound for clinical use because it causes vein irritation when administered in high concentration There is also a lag between its administration and effect *in vivo* and *in vitro* I have been hoping that the studies Dr Elliott reported would show that one of the metabolites of this compound was responsible for its anesthetic action. As you know, W D Jakoby and G. Tomkins, to whose work Dr Brodie referred, have recently shown that this compound is rapidly de-esterified and broken down to the 3-$\alpha$ and 3-$\beta$ alcohols (*Science* **123**, 940, 1956). Dr. Elliott has studied the effects of these alcohols which produce less inhibition of oxygen uptake than does the parent compound. Whether these are *the* effective metabolites is not clear, because more than one active compound is produced. Since Dr Elliott has identified two potent agents here with synergistic or additive effects, it is possible that the formation of multiple active metabolites might explain the delayed anesthetic action. The search for more possible metabolites and for other anesthetic steroids should continue From the work on "site of action," I would think also that it is not enough to screen compounds by seeing whether they inhibit oxygen uptake, as we did ten years ago, because it is quite possible that agents may be effective despite little effect on oxygen uptake. They may hit other sites of action than those Dr Elliott referred to this morning

H. W. Elliott. The two metabolites available were the $\alpha$- and $\beta$-hydroxypregnanediols and they display less inhibition at the same concentrations than does the parent compound pregnane-3-20-dione-21-ol, not the sodium hydroxydione

SEX HORMONES AND BEHAVIOR

# Genetic and Psychological Determinants of Sexual Behavior Patterns[1]

William C. Young

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Many differences in the patterns of sexual behavior displayed by individuals cannot be correlated with quantity of hormone, gonadal pathology, or abnormality of endocrine functioning (12–14, 27, 53, 54). The problem which is thereby presented has been a subject of recurrent investigation The guinea pig has been studied more intensively than any other species, but important contributory data not to be discounted have also come from experiments and observations on the rat, chimpanzee, cat, dog, and domestic fowl What has been learned from the guinea pig will be presented first, but in the interpretive portion of the review the data obtained from the other species will be given the important place that is theirs

For students of the hormones and behavior, sharp end points are as much a prerequisite as they are for workers in other fields of endocrinological investigation. Their nature, however, is totally unlike that of the end points employed in studies of tissue and organ responsiveness The end points used in work on behavior are components or elements of the total pattern of behavior which can easily be followed and quantified Each is known as a measure and together they can be used for the estimation of vigor of the behavior As would be expected, the measures of sexual behavior are different in males and females and from species to species In general the number serving as well-defined end points is proportional to the extent to which the species has been studied. No fewer than four measures are now followed in the male guinea pig and six can be distinguished in the female A number of others could be added to the lists.

In work on the male guinea pig the quantity and quality of the be-

1 A number of the investigations described in this review were aided by research grants M-504, M-504(C-C3) from the National Institute of Mental Health, National Institutes of Health, Public Health Service, and by grants from the Committee for Research in Problems of Sex, National Academy of Sciences-National Research Council

havior and latency to ejaculation expressed as a score have been used most commonly for estimation of sexual vigor (Fig. 1). Other measures are the per cent of animals in which ejaculation occurs within 10 minutes, length of the recovery period following satiation, age at the time of sexual maturation (44, 50), responsiveness to an androgen determined by the quantity of hormone necessary for the restoration of the precastrational pattern of behavior, and the time required for the organization of the neuromuscular mechanisms mediating sexual behavior (47, 48).

*The duration of heat and its reciprocal, length of the interval between* the injection of progesterone and the beginning of heat, have long provided a convenient measure of behavior in studies of the female (17).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | TOTAL BEHAVIOR | | TIME-WEIGHT FACTOR | | SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NIBBLING | | | | | | | | | | | 3 | x | .138 | = | 0 414 |
| NUZZLING | | | | | | | | | | | 11 | x | 276 | = | 3 036 |
| MOUNTING | | | | | | | | | | | 9 | x | 414 | = | 3 726 |
| INTROMISSION | | | | | | | | | | | 2 | x | .552 | = | 1 104 |
| EJACULATION | | | | | | | | | | | 1 | x | 690 | = | 0 690 |

*ONLY THE HIGHER MEASURE IS USED. TIME 29/4 MIN SCORE FOR TEST = 8 970

FIG. 1 Score-sheet used for performance of male guinea pigs in 10-minute tests (55).

Duration of the maximum lordosis is used, as are also proximity of the maximum lordosis to the beginning of heat, amount of male-like mounting, responsiveness to the conditioning injection of estrogen, and, as in the male, the time required for the organization of the tissues mediating sexual behavior (24, 25).

Each measure varies greatly, the score for the male from 0 to 20, the number of intromissions preceding ejaculation from 0 to 12 or more, the length of the interval between the beginning of a test and ejaculation 15 seconds or less to 30 minutes or more, age at the time of sexual *maturation from 63 to 96 days* Duration of heat in the female may be less than an hour or as long as 24 hours, although heat of the latter length is rare. Duration of the maximum lordosis may be 1 or 2 seconds or 30 seconds, the amount of male-like mounting from 0 to 100 or more mounts *during a single estrous period. Except for the temporal* relationship of several of the measures, a considerable degree of in-

dependence exists. Male-like mounting may be displayed at the time of estrus or it may be absent (52), the mean lordosis is longest in a strain of females least responsive to the conditioning action of estrogens (24). The result of this variability within measures, much of which is independent of the variability within other measures, is an infinite number of patterns of sexual behavior For the guinea pig and doubtless other lower mammals as well, the conclusion that differences in sexual behavior are much greater than differences in structural characters is as apt as it is for the human male and female (33, 34).

Striking to an observer of laboratory mammals is the extent to which patterns of behavior tend to be displayed consistently by individual animals. Data are most accurate for the length of heat in the female (51) and the scored behavior of males (Table I), but there are strains in which the females do not display male-like mounting and in which the duration of lordosis is characteristically long or short (24). Such differences between individuals are not related to the number of rupturing follicles (53), to the ovarian condition within the limits of the pathology encountered by Young *et al* (53), to the amount of injected α-estradiol benzoate, provided a certain threshold has been attained (Table V), or in the male, to the amount of testosterone propionate (27).

Up to the point increments of testosterone propionate have been given to male guinea pigs (27, 45), and increments of α-estradiol benzoate to females (Table II), the patterns of behavior characteristic of the precastrational period have not been altered appreciably The single exception may perhaps be latency and duration of heat in the female, but inasmuch as estrus was extended by the addition of weak responses without alteration in the intensity curves (Fig 2), the increase probably was not accompanied by a lengthening of the period of receptivity to the male.

Results of this sort gave renewed emphasis to the hypothesis advanced originally by Goodale (23) and later by Ball (2) and by Young *et al.* (53) that patterns of sexual behavior have a somatic basis, that the character of behavior displayed depends on the character of the soma or substrate on which the hormones act If the validity of this hypothesis may be assumed, the central problem for the investigator who would account for the differences between individuals is identification of the factors which influence or determine the character of the soma.

Age was shown to be involved when the responsiveness of young females to estradiol benzoate was being tested (51), and is thought to be a factor in determining the responsiveness of male tissues and organs to

TABLE I

SEXUAL BEHAVIOR SCORES OF MALE GUINEA PIGS IN SUCCESSIVE TESTS

| Animal | Tests 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 103T | 15.3 | 8.8 | 12.0 | 10.8 | 8.8 | 15.4 | 14.0 | 17.3 | 13.5 | 16.0 | 12.9 |
| 631T | 10.4 | 12.7 | 14.0 | 8.8 | 9.7 | 9.3 | 10.9 | 12.0 | 9.6 | 13.3 | 11.1 |
| 3400T | 11.1 | 10.4 | 10.5 | 9.4 | 9.8 | 11.4 | 9.0 | 9.5 | 14.7 | 12.2 | 10.8 |
| 3L | 10.0 | 8.3 | 7.0 | 6.5 | 3.5 | 7.3 | 10.3 | 6.4 | 8.7 | 4.8 | 7.3 |
| 3419L | 5.0 | 5.9 | 7.7 | 7.1 | 6.1 | 6.4 | 4.5 | 5.7 | 5.9 | 7.5 | 6.2 |
| 10L | 8.6 | 5.9 | 7.8 | 7.0 | 2.5 | 6.8 | 4.8 | 5.4 | 4.9 | 4.0 | 5.8 |
| 1599 | 2.6 | 0.6 | 3.3 | 5.3 | 4.6 | 4.3 | 2.0 | 1.8 | 1.4 | 3.8 | 2.8 |
| 611T | 0.0 | 2.6 | 3.6 | 3.3 | 2.9 | 2.6 | 2.6 | 2.4 | 2.2 | 0.9 | 2.3 |
| 144 | 1.0 | 2.6 | 3.1 | 1.8 | 1.6 | 3.0 | 1.5 | 2.0 | 0.6 | 1.9 | 1.8 |

TABLE II
RESPONSES OF 30 SPAYED FEMALE GUINEA PIGS TO DIFFERENT AMOUNTS OF α-ESTRADIOL BENZOATE AND 0 2 I U OF PROGESTERONE[a]

| | Latency in hours | Duration in hours | Maximum lordosis in seconds | Frequency of mounting |
|---|---|---|---|---|
| 50 I U. | 5 6 | 4 8 | 14.2 | 25 |
| 100 I.U. | 4 6 | 5 9 | 15 2 | 19 |
| 200 I U. | 4.3 | 6 2 | 15 3 | 27 |
| 400 I.U. | 3.7 | 7 3 | 14 7 | 25 |
| 800 I U | 3 8 | 8 0 | 14 4 | 32 |

[a] Courtesy of Dr. Robert W. Goy.

testicular androgens (30, 41, 42) Season could be a factor (3, 15, 37, 38, 40, 43), and inherent rhythms have been suggested as being influential on responsiveness to gonadal hormones (16, 18, 20, 22, 31).

Abnormal levels of thyroid hormone modify the responsiveness of the tissues mediating mating behavior in the female guinea pig (Table III), but equally striking differences are seen in euthyroid animals, so the

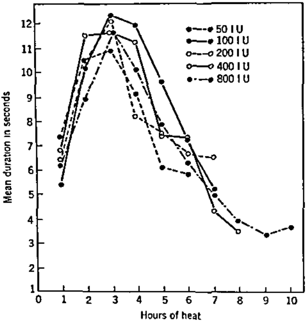


FIG 2. Intensity of lordosis on successive hours of heat in strain 2 females given different amounts of α-estradiol benzoate and 0 2 I U. of progesterone Curves end at point where 50% of females were out of heat (Courtesy of Dr. Robert W Goy )

TABLE III

THE EFFECT OF ABNORMAL THYROID LEVELS ON THE RESPONSE TO INJECTIONS OF α-ESTRADIOL BENZOATE PLUS 0.2 I.U. PROGESTERONE[a]

| Group | Amount estradiol benzoate | Number: Animals | Number: Tests | Per cent response | Average latency (hours) | Average duration (hours) | Average maximum lordosis (seconds) | Mean number full mounts | Mean number abortive mounts |
|---|---|---|---|---|---|---|---|---|---|
| Thyroxine | 100 I.U. | 14 | 21 | 85.7 (18 of 21) | 6.0 | 5.2 | 12.2 | 16.0 | 30.9 |
| Controls | 100 I.U. | 17 | 17 | 76.5 (13 of 17) | 7.4 | 4.1 | 11.5 | 8.3 | 15.6 |
| Thyroid-ectomized | 100 I.U. | 14 | 14 | 14.3 (2 of 14) | 9.0 | 3.5 | 15.5 | 6.5 | 13.0 |
| Controls | 200 I.U. | 17 | 23 | 82.6 (19 of 23) | 6.3 | 5.0 | 10.7 | 6.6 | 11.9 |
| Thyroid-ectomized | 200 I.U. | 14 | 27 | 25.9 (7 of 27) | 9.9 | 3.0 | 9.8 | 3.8 | 6.8 |
| Controls | 800 I.U. | 17 | 17 | 82.4 (14 of 17) | 8.5 | 6.1 | 10.4 | 11.6 | 10.2 |
| Thyroid-ectomized | 800 I.U. | 14 | 14 | 28.5 (4 of 14) | 9.8 | 3.8 | 6.0 | 1.2 | 2.8 |

[a] Courtesy of Doctors Robert W. Goy and Richard M. Hoar

thyroid is not believed to be involved in the differences encountered in any random selected group of females Moreover, the sexual behavior score of the male guinea pig seems to have been unaffected by the rather extreme levels of thyroid hormone that were established (56, 57). Scores of 6 9 to 7.6 were obtained from animals in which oxygen consumption in cubic centimeters per 100 grams of body weight per hour ranged from 37.3 to 80.1, and heart rate in beats per minute from 190 to 332. The sexual behavior of the male rat also appears to have been unaffected by alterations in the level of thyroid hormone Daily injections of thyroxine increased general excitability as reflected in heightened startle reflexes, but did not raise sexual excitability (6). At the other extreme, the mating performance of thyroparathyroidectomized males up to 30 days after the operation was not significantly different from that in the preoperative tests (29).

Of importance for the problem of the determination of the character of the soma is the circumstance that all the factors noted above as possibly exerting an action can easily be controlled. This was done during an inquiry into the possibility that the genetic background and contact with other animals might be effective factors.

The performance of the highly inbred strain 2 and strain 13 animals (48) and that of a genetically heterogeneous stock (for convenience referred to as strain T) has been compared. Data from intact males are summarized in Table IV. Significant differences are seen within most of the measures

For the measures for which data are sufficient to permit ranking (score, latency to ejaculation, intromissions prior to ejaculation, and per cent in which ejaculation occurred within 10 minutes), the rank-order in a descending order is strain T, 2, 13. Age at the time of sexual maturation for males of the three strains is given elsewhere (44, 49). The rank-order for this measure is also T, 2, 13.

Strain differences among the females were determined from observations made after spayed females had been injected with 100 I.U. of $\alpha$-estradiol benzoate followed 36 hours later by 0 2 I.U. of progesterone. The data are summarized in Table V The most conspicuous differences are in duration of heat, duration of maximum lordosis, and mounting. The percentage of females brought into heat is a measure of responsiveness to the estrogen. When 100 I.U. were given, the difference was not great, but when 50 I.U or 25 I U. were administered, the highly significant strain differences for this measure are readily apparent. Another

TABLE IV

SEXUAL BEHAVIOR OF MALE GUINEA PIGS FROM DIFFERENT GENETIC STRAINS

| Strain | Number (tests) | Lower Measures: Average per animal | Lower Measures: % displaying | Mountings: Average per animal | Mountings: % displaying | Intromissions: Average per animal | Intromissions: % displaying | Ejaculations: Average per animal | Ejaculations: % displaying | Average Score |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain 2 | 133 | 97.3 | 100 | 18.0 | 100 | 18.0 | 90 | 3.7 | 84 | 6.8 |
| Strain 13 | 49 | 96.4 | 100 | 6.4 | 86 | 7.6 | 57 | 0.8 | 57 | 3.0 |
| Strain T | 49 | 36.4 | 100 | 19.6 | 100 | 20.6 | 100 | 6.7 | 100 | 9.9 |

| Strain | Number (tests) | Average latency to ejaculation in 10-minute tests | Average intromissions prior to ejaculation | % in which ejaculation occurred in 10 minutes |
|---|---|---|---|---|
| Strain 2 | 405 | 6.3 | 3.9 | 28.4 |
| Strain 13 | 469 | 6.8 | 4.4 | 15.1 |
| Strain T | 213 | 4.6 | 3.2 | 73.7 |

conspicuous strain difference is in the proximity of the maximum lordosis to the beginning of heat (Fig. 3).

Unlike the situation revealed on analysis of the data from the males, is that concerning the rank-order within each measure of behavior dis-

TABLE V

SEXUAL BEHAVIOR OF FEMALE GUINEA PIGS FROM DIFFERENT GENETIC STRAINS[a]

| I.U. of α-estradiol benzoate | Strain | Number (tests) | Per cent in heat | Duration of heat in hours | Duration of maximum lordosis in seconds | Mean number of mounts |
|---|---|---|---|---|---|---|
| 100 | 2 | 42 | 98 | 7.2 | 15.6 | 0.9 |
| | T | 39 | 97 | 4.3 | 11.0 | 8.7 |
| | 13 | 51 | 90 | 5.0 | 25.2 | 37.7 |
| 50 | 2 | 14 | 100 | 6.2 | 13.9 | 2.5 |
| | T | 13 | 85 | 3.3 | 10.4 | 19.4 |
| | 13 | 17 | 53 | 4.2 | 23.9 | 46.2 |
| 25 | 2 | 14 | 41 | 6.0 | 10.3 | 0 |
| | T | 13 | 15 | 5.0 | 10.5 | 15.5 |
| | 13 | 17 | 0 | — | — | — |

[a] Courtesy of Dr Robert W. Goy

TABLE VI

ESTIMATION OF SEXUAL VIGOR IN THE FEMALE GUINEA PIG BASED ON THE RANK OF EACH STRAIN WITH RESPECT TO DURATION OF HEAT, DURATION OF MAXIMUM LORDOSIS, AND AMOUNT OF MALE-LIKE MOUNTING

| Measures | Strain 2 | Strain 13 | Strain T |
|---|---|---|---|
| A Duration of heat | 1 | 2 | 3 |
| B. Duration of maximum lordosis | 2 | 1 | 3 |
| C. Proximity of maximum lordosis to beginning of heat | 3 | 2 | 1 |
| D Responsiveness to treatment as determined by per cent brought into heat | 1 | 3 | 2 |
| E Amount of male-like mounting | 3 | 1 | 2 |

played by the females. It varies greatly and unpredictably (Table VI). The significance of this difference between males and females can only be conjectured Conceivably the measures of behavior used for the estimation of sexual vigor in the male constitute a continuum; consequently the rank-order would be the same. Perhaps in the case of the

pattern of behavior in females, several mechanisms are involved, each having its own genetic basis.

The contribution to the character of the soma which comes from contact with other animals, and is therefore experiential or psychological, has been determined for males and females of strains T, 2, and 13 (25, 47, 49).

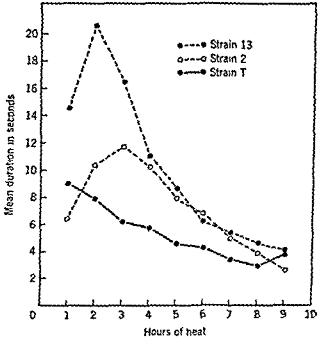


FIG. 3. Intensity of lordosis on successive hours of heat, regardless of the amount of α-estradiol benzoate (Courtesy of Dr Robert W. Goy)

A number of data obtained from the male are summarized in Table VII. The isolated males were alone with their mothers from the day of birth until day 25. Thereafter they were completely isolated The males raised in the social situation were caged with three or four animals of the same age from the day of birth until day 73 when they were isolated. The mother was removed on day 25. Both groups of males were given the first of seven weekly tests on day 77.

Examination of the data (Table VII, row 1) reveals that the performance of the socially reared strain T males was somewhat better than that of the isolated males, although none of the differences is significant. The clearest picture is given by the results from the strain 2 males in which

TABLE VII

COMPARISON OF SEXUAL BEHAVIOR OF MALE GUINEA PIGS RAISED IN ISOLATION WITH THAT OF MALES RAISED WITH FEMALES[a]

| Animals | | | Number | Lower Measures: Average per animal | Lower Measures: Per cent displaying | Mountings: Average per animal | Mountings: Per cent displaying | Intromissions: Average per animal | Intromissions: Per cent displaying | Ejaculations: Average per animal | Ejaculations: Per cent displaying | Average Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Strain T males | Isolated day 25 | 7 | 70.2 | 100 | 17.4 | 71 | 12.6 | 71 | 4.4 | 71 | 7.5 |
| | | Social situation | 7 | 36.4 | 100 | 19.6 | 100 | 20.6 | 100 | 6.7 | 100 | 9.9 |
| 2 | Strain 2 males | Isolated day 25 | 17 | 149.0 | 100 | 1.2 | 35 | 0.5 | 6 | 0.06 | 6 | 3.9 |
| | | Social situation | 19 | 97.3 | 100 | 18.0 | 100 | 18.0 | 90 | 3.7 | 84 | 6.8 |
| 3 | Strain 13 males | Isolated day 25 | 7 | 129.4 | 100 | 2.0 | 71 | 0.0 | 0 | 0.0 | 0 | 3.6 |
| | | Social situation | 7 | 96.4 | 100 | 6.4 | 86 | 7.6 | 57 | 0.6 | 57 | 3.0 |
| 4 | Strain T males | Isolated day 10 | 10 | 120.8 | 100 | 10.6 | 30 | 6.5 | 30 | 1.5 | 30 | 4.9 |
| | | Social situation | 10 | 52.9 | 100 | 14.3 | 100 | 19.6 | 100 | 5.3 | 100 | 8.1 |

[a] Data obtained from 7 tests, day 77–120, after all males were isolated. Courtesy of Doctors Elliot S. Valenstein and Walter Riss.

pattern of behavior in females, several mechanisms are involved, each having its own genetic basis

The contribution to the character of the soma which comes from contact with other animals, and is therefore experiential or psychological, has been determined for males and females of strains T, 2, and 13 (25, 47, 49).

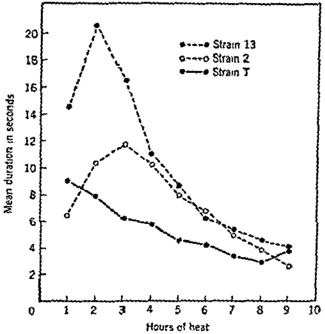


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TABLE VIII

SEXUAL BEHAVIOR OF STRAIN 2 MALE GUINEA PIGS RAISED WITH SPAYED OR INTACT FEMALES OR WITH MALES[a]

| Caging | Number | Lower measures | | Mountings | | Intromissions | | Ejaculations | | Average Score |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Average per animal | Per cent displaying | Average per animal | Per cent displaying | Average per animal | Per cent displaying | Average per animal | Per cent displaying | |
| With spayed females | 16 | 112.0 | 100 | 13.4 | 87.5 | 13.8 | 75.0 | 1.3 | 37.5 | 4.8 |
| With intact females | 10 | 94.4 | 100 | 30.8 | 100 | 25.1 | 100 | 2.8 | 80.0 | 6.5 |
| With males | 8 | 117.1 | 100 | 21.1 | 87.5 | 22.6 | 75.0 | 2.9 | 75.0 | 6.7 |

[a] Courtesy of Doctors Elliot S. Valenstein and Robert W. Goy.

the development of the measures of behavior above mounting (55) seemed dependent on contact with the other young animals (Table VII, row 2). There was some development of sexual behavior in the socially raised strain 13 males, but the level reached, although higher than that attained by the isolated males of this strain, was below that attained by the strain 2 and strain T males (Table VII, row 3).

The relatively slight difference between the isolated and socially raised strain T males was at first perplexing. Doctors Valenstein and Riss suggested, however, that this might be explained by the more rapid growth of animals in this strain. It was considered possible that many had acquired the experience they needed for the organization of the complete pattern of sexual behavior before they were weaned. The hypothesis was tested by weaning and isolating a number of males on day 10 instead of on day 25. Completely confirmatory results were obtained; mounting, intromission, ejaculation, and score were significantly lower in the isolated animals (Table VII, row 4).

In an extension of the experiment (47, 49), male cage mates were found to provide sufficient experience for the organization of sexual behavior by other males. On the other hand, males raised with spayed females performed more poorly than males of the same strain raised with intact females or males (Table VIII). The average score of the males reared with spayed females is signficantly lower than that of the males having intact female cage mates, or of the males caged with males. The results seem to stress the importance of general activity by the cage mates or perhaps mounting for the development of sexual behavior. It is frequently observed that sluggish males mounted by estrous females are stimulated to mount in return. Untreated spayed females, however, do not initiate mounting. It is considered likely, therefore, that the poorer performance of the males raised with spayed females is best explained by the lack of provocation to attempt mounting.

Presence of the testes is not necessary for the organization of sexual behavior. Two untreated strain T males castrated at birth displayed mounting proficiency before hormonal treatment, although the amount of this behavior was less than in the intact controls Subsequently, when injections of testosterone propionate were given daily, mounting increased and approached that displayed by the controls (44). Five of the six males went on to achieve ejaculation 4 to 6 weeks later.

The possibility that older males without previous opportunity to organize the higher measures of sexual behavior could do so by means of contact with other animals was investigated (47) Strain T males which

TABLE IX
SEXUAL BEHAVIOR OF FEMALE GUINEA PIGS[a]

| Caging situation prior to tests | Strain | Number: Animals | Number: Tests | Per cent response | Mean latency of heat in hours | Mean duration of heat in hours | Mean maximum lordosis in seconds | % of heat periods when full mounts were exhibited | Mean no full mounts per test | Mean no abortive mounts per test |
|---|---|---|---|---|---|---|---|---|---|---|
| Social from birth to day 180 | T | 11 | 55 | 85 | 6.1 | 4.9 | 12.3 | 34 | 3.5 | 9.3 |
| | 2 | 10 | 10 | 100 | 3.9 | 7.9 | 13.8 | 0 | 0.0 | 1.8 |
| | 13 | 10 | 48 | 78 | 6.4 | 4.8 | 21.8 | 42 | 1.7 | 16.2 |
| Isolated from birth to day 150 | T | 10 | 40 | 80 | 8.3 | 3.4 | 6.9 | 25 | 0.8 | 2.0 |
| | 2 | 10 | 10 | 100 | 4.6 | 5.9 | 9.3 | 0 | 0.0 | 1.9 |
| | 13 | 10 | 40 | 60 | 7.5 | 2.8 | 11.0 | 0 | 0.0 | 3.5 |
| Isolated from birth to day 150, social from day 240 to 300 | T | 5 | 10 | 90 | 6.4 | 4.0 | 11.5 | 22 | 1.3 | 1.7 |
| | 2 | 10 | 10 | 100 | 4.9 | 6.8 | 11.2 | 10 | 0.1 | 3.9 |
| | 13 | 5 | 10 | 60 | 6.6 | 2.6 | 17.6 | 20 | 0.2 | 4.8 |

[a] Comparison of sexual behavior of female guinea pigs spayed at birth and raised in isolation (1) with that of females spayed at birth and raised with other animals, and (2) with that displayed by females spayed at birth and given contact with other animals after adulthood had been attained. Tests were made following the injection of 100 I.U. of $\alpha$-estradiol benzoate followed by 0.2 I.U. of progesterone. Courtesy of Dr. Robert W. Goy.

had not exhibited any of the more mature behavior in tests given while they were isolated were each placed with two females for 23 days when they were 320 to 430 days of age. In subsequent tests all these males mounted, had intromissions, and ejaculated. Strain 2 males of approximately the same age did not acquire the copulatory pattern so readily. Two of five achieved intromission and ejaculation after having been with females, but none of the remaining three displayed any of the higher measures of sexual behavior. The data indicate that the organization of sexual behavior patterns in the male guinea pig is not as sharply restricted to an early critical period as is the behavior described in the literature dealing with the phenomenon of imprinting in birds (36). It is clear though that contact with other animals is not as effective in organizing the tissues mediating mating behavior in older animals as in young males.

Data were obtained from comparable experiments on the female Animals from the three strains were spayed within 5 days of birth and raised either in isolation or in a social group. An isolated female was with her mother until day 25; thereafter she was alone in a cage Females reared socially were caged with their siblings until day 25 when the mother was removed. Thereafter they were confined with two to ten females and two or three males. This manner of caging was not changed during the remainder of the investigation except that on the days tests were made the animals were placed together, but the isolated and socially raised females were always in different observation cages.

The results from tests made following the administration of 100 I U. of α-estradiol benzoate and 0.2 I.U. of progesterone are summarized in Table IX. In general the behavior was quantitatively less for the isolated females than for those raised socially The most conspicuous exception involved the strain 2 animals Even when females from this strain are raised socially, they do not mount, reduction following isolation therefore is impossible. As in the experiments on the males, the importance of the genetic background is evident The vigorously mounting strain 13 females require contact with other animals for the organization of the nervous tissues mediating this behavior, whereas no amount of contact with other animals stimulates the display of mounting by strain 2 females.

These experiments in which females spayed within 5 days after birth were used revealed that the ovaries are not necessary for the organizing action which occurs when there is contact with other animals In another experiment females were isolated from birth, but spayed as adults

or aggression by a female, however, can have a depressing effect (9). Castration of the male cat is followed by the loss of sexual behavior with copulatory responses dropping out first, followed later by the loss of mounting behavior. On the other hand, sexual experience before castration retards the loss of sexual behavior for periods up to two and one-half years (46).

The development of sexual behavior patterns in the chimpanzee has been studied by Nissen (39). Males and females after having been raised in a nursery for 2 to 3 years, were transferred to cages where they lived in sex-mixed groups. Before puberty the sexes were separated and placed in adjacent cages Beginning well after puberty the animals were paired in male-female combinations in such a way that the younger inexperienced animal was with an older experienced animal of the opposite sex. In many hundreds of sessions the complete mating pattern was not shown, although almost all the component or unit acts of the pattern were displayed With only a slight modification of Nissen's words, the component acts are either innate or they are learned in early life without the act of learning being observed. The complete sequential pattern of mating behavior, on the other hand, is not innately determined, or at best, the innate factor is only a readiness or predisposition to learn. Several factors increase or decrease the probability that such learning will occur. Foremost is the activity level The greater the activity and the more it is varied, the more likely is the development of the complete pattern. Age is critical. As the chimpanzee matures, the amount of activity in which he engages decreases With this slowing up, the probability of there being the concatenation mentioned above is reduced

If what has been found for the chicken, guinea pig, dog, cat, and chimpanzee is to be the basis for the hypothesis that patterns of sexual behavior are learned rather than being wholly innate, what has been reported for the rat is enigmatic. The results from an early study of rats raised in isolation, cohabitation, and segregation (4), and from a more recent investigation in which different kinds of experience were given (32) are consistent in leading to the conclusion that the complete pattern of sexual behavior including ejaculation was displayed more frequently by the males raised in isolation The data are taken to indicate that patterns of social behavior formed before complete mating was physically possible tend to persist and to inhibit the normal sexual responses.

Evidence for an organizing action in the positive sense has not been found. In an unpublished experiment described by Beach (11) male rats

The results were similar to those described above. Clearly, therefore, presence of the ovaries from birth to day 120 does not compensate for the effects of isolation.

In a final experiment with the female contact with other animals was not provided until day 240 Duration of heat, maximum lordosis, and mounting were less than in the females raised socially from the day of birth (Table IX). The difference between the groups was even more striking than in the males.

The conclusions which may be deduced from these experiments on male and female guinea pigs are summarized: (1) Contact with other animals is important if not necessary for the development or organization of the complete pattern of sexual behavior. (2) The extent to which the psychological or experiential factor influences the organization of such behavior depends on the genetic background. At the moment, relative importance may not be assigned to either factor. In general the adult pattern has not appeared in the absence of contact with other animals At the same time no experience provided in the experiments which have been reviewed was sufficient to eliminate the between-strain differences. (3) The experiential factor is a more effective stimulus to the maturation of patterns of sexual behavior when the animals are young. (4) In neither sex is the presence of the gonads necessary for the action of this factor (5) The element in the contact with other animals which is most important for the organization of sexual behavior has not been identified. In the case of the male a certain amount of general activity and perhaps mounting by the cage mates appears to be important.

Generalization with respect to the factors which are influential in determining the character of the soma would be hazardous if our data had been obtained from only one species. Fortunately, however, supplementary information has come from investigations of several species. Some bears on influences transitory in their effects, such as the inhibition strange surroundings may have on the behavior leading to copulation and ejaculation, but much has to do with influences exerting lasting effects It is with the latter we are concerned

In the absence of other males white Leghorn cocks tend to be consistent in the frequency of matings. When, however, four cocks raised together for about 3 months were placed in a pen containing seven hens, a suppression developed which persisted after the dominant cock was removed (28). Satisfactory sexual relations are placed high on the list of experiences which augment the sexual interest of male dogs Attack

Beyond bringing the character of the soma to expression, the role of gonadal hormones is not clear, even in lower mammals. It is not sufficient to conclude that they are excitatory in the sense of stimulating sexual arousal (7, 8, 10). In their absence sexually experienced male rats and guinea pigs display a nondirected hyperexcitability (5, 26). When androgens are administered to such animals the excitability is not necessarily increased, but it is channeled in the direction of successful copulation.

In man the inadequacy of our knowledge is even more apparent. The organization of the substrate may have been brought so close to an autonomous expression of sexual behavior by innate factors and learning that relatively little gonadal hormone is required. An analogy might be found in the striated muscles and seminal vesicles of a castrated male. Some muscles are smaller and perhaps less effective but they still function (35) The seminal vesicles, on the contrary, have shrunk to a mere vestige and are completely nonfunctional. From the standpoint of function, dependence of muscles on the hormone is relatively slight, dependence of the seminal vesicles relatively great. If a difference of this sort exists between the neuromuscular tissues mediating sexual behavior in man and the corresponding tissues in lower mammals, it would help account for the seeming lack of relationship between the gonadal hormones and patterns of sexual behavior in man

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were isolated on day 14 just when the eyes were opening and before they could have had experience mounting their cage mates or being mounted. Between days 90 and 100, thirteen experimental animals and twelve siblings which had been reared together were tested. In the first 10-minute test four of the experimental and five of the control males mated The copulatory performance is reported to have been perfect. Latencies were 195, 180, 60, and 8 seconds in the experimentals and 110, 105, 75, 40, and 25 seconds in the controls We cannot account for the difference between the guinea pig and chimpanzee, on the one hand, and the rat, on the other, unless it is to suggest that in the evolution of the rat the organization of the mechanisms mediating mating behavior has become innate very much as the evolution of the cowbird has been accompanied by the development of a precocious behavior which gives the young competitive advantage in the nest of the parasitized species (21).

Whatever interpretation is placed on the data obtained from the rat, the evidence seems incontrovertible that in two species widely separated phylogenetically the character of the tissues mediating sexual behavior in males and females is strongly influenced by experiential factors. Presumptive evidence exists that such factors are also important for the organization of sexual behavior patterns in male chickens, dogs, and cats

The fortuitous availability of inbred strains of guinea pigs has enabled us to demonstrate something of the importance of the genetic background. Limitations are imposed which neither the experiential factor nor any action of gonadal hormones has overcome. But by the same token, the genetic background does not have an autonomous role in determining the character of the soma. Contact with other animals is necessary if the genetically determined "portion" of the substrate is to be readied for expression by the subsequent action of a hormone

The point of view we have developed provides an explanation for the opinion that gonadal hormones are without effect on sexual behavior (34), and why specificity of their actions, discussed at length by Antliff and Young (1), has been questioned (8, 19, 34). The hormones act on a substrate or soma already established and bring out just what is there. In the case of the female guinea pig this may be male-like mounting without estrus, it may be a vigorous lordosis without mounting, or it may be any one of an infinite number of intermediate patterns. In some males ejaculation may be achieved within 5 minutes, in others 20 minutes or more may be required.

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## Discussion

CHAIRMAN R W GERARD Thank you, Dr Young, for a very excellent presentation

A C GOLDSTEIN In your experiment with the animals raised in social isolation and in social conditions have you investigated the metabolic differences in these animals before you tested them? I am trying to get at possible mechanisms for this

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A C GOLDSTEIN Do you intend to raise the animals, maintain them, and furnish them with some experience such as mounting various objects?

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D. W WOOLLEY· I want to raise a point about this social effect also We have been doing experiments overcoming the psychotic behavior of mice given lysergic acid diethylamide (LSD) by administration of serotonin plus cholinergic substances One can overcome the LSD by injection of the serotonin and cholinergic substance into the lateral ventricle. We have found in our experiments that the social experience is quite important If one goes to the mouse colony and selects animals from a dozen cages and combines them in one box, so that strangers are together, it takes about a quarter as much of the cholinergic agent to be effective *in these animals as it does if the animals have been housed individually* for 24–48 hours The animals become mixed up in a new social atmosphere. The variability in their response is much greater than those kept isolated

W. C YOUNG We have tried administering larger amounts of testosterone to the males brought up under conditions of isolation and the larger amounts of testosterone were not effective. Dr Goy has also injected large amounts of estrogen into females. In neither sex does the hormone substitute for lack of contact with other animals

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C. G. HARTMAN: Going up in the scale a bit, I might mention the case of a male monkey which had been raised in isolation—a very handsome fellow, who was not worth a damn as a breeder. He did not seem to know what heterosexual relations were all about; he would show some sexual excitement by masturbating, but he never copulated.

Well, Dr. Young, I am sure you know from your stay at Yerkes Chimpanzee Colony in Orange Park that for that species generalized play of the immature chimp is an important prelude to sexual maturation, behaviorally speaking.

W. C. YOUNG: Henry Niesen has beautiful data on that

G. PINCUS Do you have knowledge about the function of the adrenal steroids? Have you done work on those?

W. C. YOUNG: No. If you people could teach me how to adrenalectomize a guinea pig and keep it alive, I would be delighted.

G. PINCUS. The method has been published but they live for only a few days

R. A. CLEGHORN Cannot they be sustained?

G. PINCUS. It is the most difficult animal in our experience except possibly the rabbit. They are equally bad but you could of course use adrenal steroids to offset the adrenalectomy effect I am wondering whether the question of drive that you call genetic might not be accountable to some other endocrine.

W. C. YOUNG. We certainly cannot eliminate that possibility, although in the female again with such a mixture in the order of these things I am inclined to think it is basically genetic.

CHAIRMAN R W GERARD· I am afraid that does not mean much "Basically genetic," by the time you are making a test on the animal, merely means the animal is different. At that time the geneticists would say how it got that way, but there would also be at that moment a difference in hormones, in brain, or in some other part of the organism.

W. C. YOUNG. We think it is not a difference in gonadal hormones. We are controlling the amount by using gonadectomized animals and the same quantities of injected hormone

CHAIRMAN R. W. GERARD. Since I have got the floor for a moment, have you ever made an effort to even look grossly at the brains of these high, medium, and low performers to see if there is a different relative development of the relevant different parts?

W. C YOUNG· No

CHAIRMAN R. W. GERARD This is the kind of thing that might easily relate to a differential activity of various brain regions

W. C. YOUNG: Yes, we hope to get into something like that as the next step

CHAIRMAN R. W GERARD. Make some quick frozen sections, even with camera lucida outlines, and see if you get any major clues that way before you go in for an enormous number of serial sections.

E. ANDERSON. What area of the brain?

CHAIRMAN R. W. GERARD Now you are expecting me to be an anatomist At that stage, I think the obvious ones

G. S. GORDAN· Which are obvious?

CHAIRMAN R W GERARD: Anything under the cortex

R G Hoskins· I merely want to ask if there is any work on the effect of the social factor on the age at which sexuality begins to appear.

W. C. Young: Yes, there is. In animals brought up under conditions of isolation, sexual maturation is relatively slow.

C. G Hartman. There are other studies on that, are there not? On mice and rats, I believe.

W. C Young: I am not aware of them, but precocious development has been reported by Calvin Stone and Frank Beech following the injection of androgenic substances into young males

B. B Brodie. What are your thoughts on the reason for the sedation that follows from the sexual act which seems much greater than would be expected from the expenditure of energy?

W. C Young· I have no thoughts except what I mentioned to you over the luncheon table that this sedation can be overcome by a sufficiently strong psychological stimulus Typically, the male guinea pig ejaculates once and goes off into the corner and is quiet for some time We observed these animals for about 60 minutes and gave up We then modified things a bit We removed the female at the end of 30 minutes, dropped her into a basket, picked her up and replaced her in the cage. The male continued to lie in his corner When, however, instead of replacing her at the end of 30 minutes, we replaced her with a second female in heat, his interest was somewhat revived. One male even displayed a second ejaculation in the second 30-minute period, and in every case I believe there was considerably more activity stimulated by the second female than by the first.

C G Hartman I would like to call Dr Brodie's attention, if I may, to the fact that a ram may copulate with 50 ewes in a single day, producing two billion sperms at each ejaculate and pregnating 48 of the 50 ewes A rabbit buck has been known to sire 40 litters in one day. These males must have some mechanism for quick recovery of nervous energy.

B B Brodie But there must be some reason for the sedation observed in some species

Chairman R W. Gerard You are confusing cathectic energy with physical energy which is one of the mistakes that has been made over the decades

F F Flach. Did you say there was a difference in the effectiveness of socialization in grouping in one strain and not in the other?

W C Young Yes

uck by in the first paper the amygdaloid nucleus responded to the experiment while dogs did not I have always been, in an informal way, struck by the schizoid quality of cats I don't want to offend anyone who is a cat lover, but from my observations cats usually form tenuous relationships with humans They remain aloof from the beginning to the end of their existence in the family and are prone to sudden rage reactions On the other hand, dogs often identify with people in the family constellation and will form more complex emotional relationships Carried over into human terms, this poses a question Why not investigate the high or low degree of drive and particularly those factors which

explain the difference? Similar differences occur in human beings and it may well be that by analyzing these species-specific factors in animals we may obtain a clue as to why in a particular person, say a schizophrenic type, not only the personality structure but also the metabolic setup, might react in unusual ways to otherwise relatively normal substances.

G. H. GLASER. Dr. William Scoville, of Hartford, has reported to us observations concerning bilateral ablations of the hippocampus in human schizophrenics as an experimental therapeutic procedure. These individuals did not show overactive hypersexual behavior. In fact they showed a lessening of drive

A. C GOLDSTEIN. In a recent paper by L Schreiner and A Kling (*Am. J Physiol* 184, 486, 1956) amygdalectomy in different species produced more uniform changes on emotional behavior than on sex behavior, so that it would apparently be wrong to conclude that strong sexual manifestations necessarily follow amygdalectomy.

In the matter of the period of sluggishness that follows ejaculation there are, as Dr. Hartman mentioned, very wide differences among species but in the rat interesting things happen. This has been studied rather more extensively in the rat. We have let rats mate as long as they wished during a period of a day and they will go at times up to nine consecutive ejaculations over a period of hours and in this period the most consistent effect from ejaculation to ejaculation is a lengthening of refractory period Even in an individual animal that is quite consistent, it lengthens from ejaculation to ejaculation On the mechanism of it very little is known

W. C. YOUNG. Just one comment I should add that relatively few of us have worked in this field, at least over a period of years To be sure, there have been many observations, but I think that the group at Yale and the group at Kansas have done more work than has been done in other laboratories. Fortunately Frank Beach and I have recently been able to compare notes and compile a list of species differences in the rat and guinea pig If confidence in the observer is assumed, the striking thing is the number of species differences and the radical nature of the differences, and both feel, I believe, that before general interpretation can be made we must have information from many other species

CHAIRMAN R. W GERARD Just to follow that up with a comment that I did not expect to make, Sherry Washburn was talking to me recently after coming back from Africa where he practically lived with a number of baboon groups. He says the attitude of the senior male towards the younger male as regards possession of the female, is entirely different than universally assumed There seems to be no concern about it at all, they just rotate around freely

# The Experimental Control of Sex Behavior in Animals

ALLAN C. GOLDSTEIN

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The experimental control of sex behavior in animals is a vast topic, and I have restricted the material in two ways in order to give some unity to this presentation in the time available. First, I have drawn most of the following material from the literature on mammalian sex behavior. This is not, however, a serious drawback since the best studied examples are mammalian. Second, I have emphasized for the purposes of this conference the neural and endocrine mechanisms in sex behavior. However, I do not want this to leave the impression that sexual behavior is "nothing but" the neural and endocrine mechanisms, for there are many other classes of events which we could, with justification, speak of as controlling sex behavior. In many species, for example, especially among seasonally breeding forms, gradual endocrine changes prepare the animal for the breeding season. These endocrine changes are often precipitated by environmental factors such as the amount of daily light, the amount of food available, or the climatic conditions. The act of mating itself may be induced by other environmental events, such as the presence of the mating partner, or the presence of a nesting area. Also, sex behavior can be greatly modified by the effects of previous experience (21, 53), and one aspect of this problem is treated in Dr Young's paper. I shall not be discussing these methods of control, but it is worthwhile to keep in mind that these factors can be as important to mating behavior as those internal events which we classify as neural and hormonal.

In addition to illustrating some main points in our knowledge of the neuroendocrine mechanisms of sex behavior, I shall supplement these with an account of some work which has recently been completed in the Yale laboratories of Dr. Frank Beach. Much of this work is still in press or is being prepared for publication.

To make the discussion more concrete, the following is a typical mating pattern in the four-legged mammal. In the female the fundamental copulatory response consists of a flattening or depressing of the back, with the result that the perineum is elevated and exposed. This is the lordosis response. In those species where it is necessary the tail is

moved laterally. In the mating behavior of the male, the pattern consists of mounting the female from the rear with the forelegs resting on her back or gripping her sides. The male then executes thrusts of the hindquarters until intromission occurs. If intromission does not occur, the male dismounts and tries again a short time later. In some species intromission occurs once and is prolonged until the male ejaculates. This is what happens in the cat and dog. In other species, such as the rat, the male may mount, achieve intromission, and dismount several times before ejaculation occurs (19, p. 251).

## I. ENDOCRINE FACTORS

### 1 Effects of Castration in the Male

In the male rat (24, 62), guinea pig (40, 60) and hamster (25) characteristic changes occur after the male is castrated. The ejaculatory response is the first element to disappear. Next the ability to gain intromission decreases in frequency or disappears. However, in these species the castrate male may still become excited when placed with the estrous female, and may chase and sniff at her hindquarters Figure 1 shows data from the hamster and illustrates the fall in the copulatory response. Figure 2 presents data from the guinea pig, and is taken from a paper by Grunt and Young (40). They used a measure of sex behavior which is a composite score based on a weighting of all the elements of the mating pattern (66). Since it takes into account the sniffing and the following which persist after castration, their score does not fall to zero

Recent work at the Yale laboratories has furnished comparable data for the dog. The results are now being prepared for publication in a series of papers and represent work carried out over eight years on various aspects of mating behavior in mongrels and purebred beagles. The dog is an interesting animal for this work because, unlike the rat, the guinea pig, and the hamster, its cerebral cortex is convoluted and its social patterns are more highly developed At present the results indicate fairly definite differences between how dogs respond to castration and what has been previously seen in the smaller mammals.

Figures 3 and 4 show the individual records of several dogs. Nine dogs were used in this experiment. five were mongrels and four were beagles Two of the dogs were castrated before puberty and observed thereafter The seven dogs whose records are discussed here were adults when castrated They were studied for at least several months before castration and for one to five years after castration

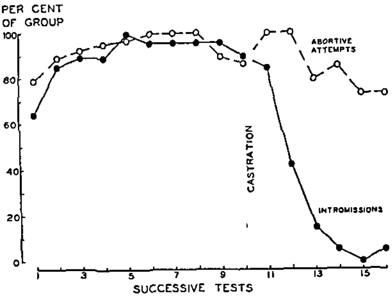


FIG. 1. Effect of castration on the mating behavior of the male golden hamster. After castration the per cent of the group that showed at least one intromission per test steadily declined although there was no change in the per cent of the group showing abortive attempts to gain intromission. (25, p 216, Fig 1 )

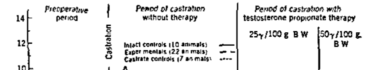


FIG 2 Effects of castration on the mating behavior of the male guinea pig After castration there was a steady decrease in the sex score (see text) which returned to normal after treatment with androgen. (40, p 241, Fig. 1.)

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in the mating sequence, whereas the shorter ones do not. In the latter case, dogs terminate the intromission and very shortly thereafter begin to mate again. The tests on which the dogs maintained intromission for longer than 4 minutes are called "lock" tests to distinguish them. In the scoring system used, the animal was judged to have shown the complete mating pattern only on tests in which locks occurred.

There were three classes of response to castration appearing in the individual dogs. In three dogs there was a consistent decline in the per

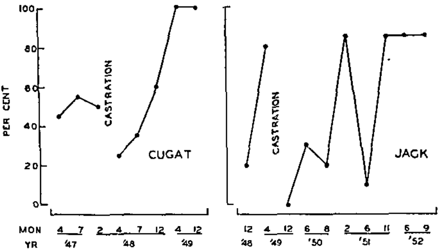


FIG 4 Effects of castration in the male dog Plotted as in Fig 3 In these two dogs there was an initial decline in the per cent of tests in each series in which the complete mating pattern was seen, but with time their performance surpassed that seen before castration

cent of tests in which the complete mating pattern was seen This decline is the typical response to castration seen in rodents (The non-zero tests for the dog Gundy are tests on which androgen was given This is discussed in more detail later ) Two dogs showed no important change in their behavior after the initial drop common to all the animals immediately after operation The later performance of these dogs approximated their performance before castration. The remaining two dogs' sex behavior improved after castration, until they showed the complete mating pattern on a higher per cent of tests than they had before castration This effect differs from castrational changes seen in lower mammals and constitutes an effect whose explanation is not at all clear.

When male dogs gain intromission, the penis usually becomes engorged so that it cannot be withdrawn for a period of time, about 10 minutes on the average. According to normative data on the dogs there is a bimodal distribution of these intromission times. The distribution peaks once for times less than 4 minutes, and once for times longer than 4 minutes. These longer intromission times function as a terminal event

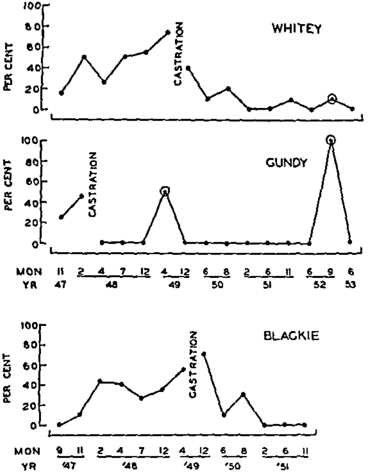


FIG. 3. Effects of castration on the mating behavior of the male dog. Each point represents a series of mating tests In each of the three dogs there was a decline after castration in the per cent of tests within each series in which the complete mating pattern was seen The points which have been circled represent tests in which the dogs received injections of androgen The date of the test series appears on the abscissa.

ance of the complete pattern depends upon amounts of androgens being present.

In the above studies, castrates were usually given a constant amount of hormone daily and the return of mating behavior noted The relationship between the amount of hormone given daily and the return of behavior has also been studied. Most of this work has been done with male animals, because the male mating pattern of guinea pigs and rats, the species which have been studied, has been easier to quantify satisfactorily than has the mating pattern of the females.

A study by Beach and Holz-Tucker (24) determined the minimal amounts of hormone necessary to maintain sex behavior in castrate male rats. The animals were given weekly mating tests before and after castration. It was found that 50 to 75 $\mu$g of testosterone propionate daily maintained the animals at preoperative levels. Figure 5 shows the positive relationship between the per cent of animals ejaculating in a 10-minute test and the amount of hormone given daily. After the fifteenth week, the hormone levels were changed. The effectiveness of the 75 $\mu$g. dose can be seen here In Fig. 6 are presented data on the copulatory response Some animals continued to mount and gain intromission several weeks after castration. This effect was not always due to the fact that a few animals were resistant to the effects of castration, since many animals failed to copulate for several weeks during castration and then copulated on a following test

In the guinea pig, a similar study by Grunt and Young (40, 41) has shown that once the minimum daily dose which will maintain sex behavior in castrates at the preoperative level is surpassed, there is no further increase in sex behavior This differs from the finding of Beach and Holz-Tucker, where there was an improvement in certain measures of sex behavior when hormone was given in quantities greater than the maintenance dose

The differences between the results of Beach and Holz-Tucker (24) and Grunt and Young (40) are interesting. In these experiments and in several others with rats and guinea pigs, there are suggestions of specific differences in the behavioral response to hormones and experience. It is good to keep these differences in mind, since many discussions of sex behavior tend to look upon the sex behavior of all but the primates and man as essentially similar. We can probably expect to find more and more specific differences among mammals with more detailed experimentation.

In all animals there was a high correlation between the number of sex tests conducted before castration and the level of sex behavior seen in the series of tests immediately after castration (+ 0.83, $p < 0.05$). This means that the more experience the animals had before castration, the less severely their behavior was affected on the tests immediately after castration. But we have not been able to find any variable systematically related to the long range pattern of behavior shown after castration.

The most important effect of castration was the decreased ability of the males to lock with the females. There was no difference in the per cent of tests on which intact and castrated males mounted the female There was a slight, unreliable decrease in the per cent of tests in which intromission occurred, and when intromission was achieved it was not maintained so long as in the intact dog But the major effect was the inability of the castrated dogs to lock with the female, with the result that there was a decrease in the per cent of tests on which the complete mating pattern was seen In the two dogs in which sex behavior improved *during the castration period, this improvement was chiefly* due to increased ability to lock with the female.

Testosterone propionate was given to three castrates. The points circled in Fig. 3 are tests on which hormone was given. There was an increase in the per cent of tests on which the complete pattern was seen These animals had previously locked on 8% of the tests on which they achieved intromission During testosterone treatment this figure rose to 71%

## 2. Effects of Androgens in Castrate Male Animals

In castrate males of other species, treatment with androgenic substances is followed by a return of the separate elements of the mating pattern in approximately the reverse order in which they disappeared after castration. Castrate male rats (23, 62) and guinea pigs (40, 41) after injection of androgen showed increased excitement in the presence of the female and increased frequencies of mounting and gaining intromission There was also an increased per cent of males ejaculating during the mating tests. This is also the approximate order in which the elements of mating appear about the time of puberty (61) Although the complete mating pattern is not seen until after puberty, some mounting behavior is observed in prepuberal males (15a) It seems reasonable, therefore, that certain elements of the male mating pattern can function in the presence of little or no testicular secretions although the appear-

### 3 Effects of Castration and Hormone Replacement in the Female

Although there have been no quantitative studies of the female behavior before and after castration, the evidence that there is pertaining to female rats (13), guinea pigs (67), hamsters (45), and cats (6, 68) suggests that there is a complete cessation of the periods of female receptivity after bilateral ovariectomy. The females vigorously resist sexual advances by the male, and less willingly tolerate bodily contact. This is to be contrasted with the gradual loss of mating behavior typically found in the male, in which behavior persists some time after castration.

It has been known since the work of Long and Evans (49), Wang (63), and Slonaker (59) that heat behavior in the female rat is associated with a vaginal smear characterizing the time when the graafian follicles are enlarging within the ovaries. Estrogenic substances are being liberated at these times In the cat (68) and dog (51, and our observations) estrogen injected alone will induce heat in the anestrous or spayed female In the dog, this induced heat, judged by the effects on the male, closely approximates the naturally occurring heat behavior (our observations). In the rat (13) and guinea pig (33) on the other hand, only a small per cent of females are brought into heat by estrogen alone. But when estrogen is followed by injection of progesterone at the appropriate time, a large proportion of females come into heat In the rat this induced heat is indistinguishable from the naturally occurring behavior (16).

Kislak and Beach (45) have reported an interesting relationship between these hormones and heat behavior. The female hamster is an extremely aggressive animal except when it is in heat. If a female and a male are confined together in a cage when the female is not sexually receptive, the female will maim, and occasionally kill the male. Kislak and Beach found that this aggressiveness was inhibited by the same hormonal conditions which induced heat in the female. Neither estrogen nor progesterone alone induced heat Neither affected aggressiveness. When both were injected at the proper times, the female came into heat and aggressiveness was practically eliminated. This relationship between aggressiveness and sex hormones is not specific to the hamster. Schreiner and Kling (56) found that large injections of diethylstilbestrol, a synthetic estrogen, produced aggressiveness and irritability in two young, previously docile female cats. Variations in aggressiveness during the estrous cycle have also been reported for the guinea pig (64) and rat (17).

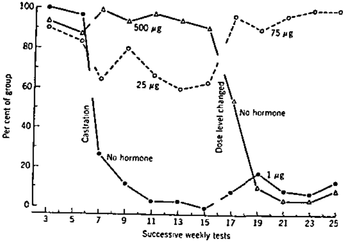


FIG 5. Effects of different amounts of testosterone propionate on the mating behavior of castrated male rats Percentage of each group ejaculating at least once during the mating test (24, p. 445, Fig 4 )

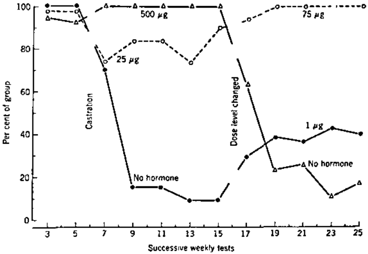


FIG. 6 Effects of different amounts of testosterone propionate on the mating behavior of castrated male rats Percentage of each group showing the copulatory response (intromission) at least once during the mating test (24, p 445, Fig 4 )

(65), in rats it occurs throughout the entire cycle (26). It is abolished by ovariectomy in the guinea pig (65), but persists in the rat (14, 26).

One method which has been employed in studying the mechanism of this bisexual behavior has been by injecting the heterologous sex hormones. Ball (3) injected estrin into male rats which had been castrated two months earlier This estrogenic substance increased the level of male behavior above the castrate level. Even the ejaculatory response, which is quite rare two months after castration, was occasionally seen In another study, Ball (4) castrated male and female rats at weaning and treated them with estrogenic substances for 1½ to 3 months. Animals of both sexes showed lordosis, but this was more difficult to elicit in males. With adult female rats, Ball (5) found that injections of testosterone abolished the vaginal cycles and increased the incidence of male behavior over that usually seen. These studies have been confirmed and extended by Beach (14, 15).

Beach reported the interesting finding (11) that intact male rats treated with testosterone propionate continued to show typical male behavior until they had received 13 to 23 mg androgen. At this dosage five of eight males showed all the elements of the female pattern: hopping, ear-wiggling, and lordosis. These responses were difficult to elicit and sluggish

These findings taken together permit certain conclusions and also serve to summarize much of our knowledge of the endocrinological basis of mating in lower mammals. Sex hormones are normally quite specific in their influence on behavior Androgens, whether given to castrate male or female animals, typically increase male behavior. Estrogen (with progesterone where needed) typically increases female behavior. There are limits to their specificity, however Hormones can at times facilitate in the same animal both the male and female mating patterns, but there seems to be a threshold effect. The native pattern occurs with a lower threshold It may be that these hormones have both a general and a specific effect on behavior. on the one hand there may be the general effect which facilitates both sex patterns, and on the other, the specific *effect which acts primarily on the native pattern*

To return to our original problem of bisexual behavior, this hypothesis seems to clarify some things. In the high drive male which shows female behavior (18) and in the female guinea pig which shows mounting behavior at estrus (65), the above hypothesis is satisfactory since it suggests that high sex drive facilitates both mating patterns. But in the female rat in which mounting occurs after ovariectomy (26) the problem still

## 4. The Specificity of Mating Patterns

The above studies show clearly that androgens are indispensable for the full mating pattern to occur in the castrate male, and that estrogens, alone or in combination with progesterone, are indispensable for the full mating pattern to occur in spayed or anestrous females. Relatively little attention has been given, however, to the effects of androgens on female animals and estrogens on male animals. This information has great relevance to understanding the mechanisms of sex behavior.

First, however, one must recognize the amply documented observations that there is no invariable sexual specificity in mating behavior. The predominant finding in lower animals is that male behavior is restricted to male animals and female behavior to female animals, but this is not absolute Female rats (9, 12, 14) and guinea pigs (65) and females of several other species show some elements of the male mating pattern, especially mounting behavior. In female rats the entire male pattern (excluding, of course, the ejaculatory response) is occasionally seen, including the copulatory response with its distinctive backward lunge (12). The possibility that this male behavior is due to ovarian androgens does not appear likely. Young and Rundlett (67) working with ovariectomized guinea pigs found that estrogen and progesterone in combination elicited mounting behavior in these animals These injected hormones rule out any effects from ovarian androgen, and indeed these authors found that these hormones were even more effective than injected androgen Beach and Rasquin (26) found that mounting activity occurred in female rats ovariectomized either before or after puberty. It therefore seems highly unlikely that this mounting behavior in the female can be related to ovarian androgens.

Female responses are known to occur in some males. Beach (18) has reported one male rat which displayed very vigorous male mating responses when with females, but also showed the complete female mating pattern when it was mounted by other males When the animal was castrated there was an abrupt loss of the female pattern and a gradual loss of the male pattern. Injections of testosterone propionate restored both the male and the female patterns. Subsequent injections of estrogen and progesterone restored both patterns, but the male ejaculatory response was absent.

These studies demonstrate that the potentiality for both male and female behavior exists in both sexes Its hormonal basis is not at all consistent since in female guinea pigs, mounting occurs only at estrus

necessary. Pauker (52) was unable to find any significant effect on the mating behavior of male hamsters after removal of these structures

In female rats surgical removal of the uterus and vagina did not abolish mating (2), and in the rabbit deafferentation of the genital region did not prevent mating (31).

The above studies show that genital sensitivity is not essential for the initiation and maintenance of sex behavior. There is evidence to indicate that it has some importance in this respect. Ball (2) found that the responses diminished in strength in the absence of genital stimulation. On the positive side, observational evidence (39, Chapt. 3) points up many instances, especially among primates, in which genital stimulation is a significant part of sexual play. Just how much importance should be attached to this cannot be judged at present.

### 2 Diencephalon and Hypothalamus

An early study to implicate the diencephalon in mating was that of Dempsey and Rioch (34). They transected at different levels of the guinea pig brain and found an area in the vicinity of the mammillary bodies which was essential to the female's estrous responses. Dey *et al* (36) found that guinea pigs with lesions in the anterior hypothalamus also failed to show estrous responses when mounted by males They brought forth evidence to show that this deficit was probably not due to gonadotropic involvement.

Two later studies confirmed that the effect described by Dey *et al.* (36) was not due to involvement of the gonadotropic function of the pituitary Brookhart *et al* (30) determined the amount of hormone which would bring a female guinea pig into heat They then placed lesions in the anterior hypothalamus and found that the previous hormone dosages, or two to four times the amount, were inadequate to bring the animals into heat. Dey *et al* (37) placed lesions, which were the size of the hypothalamic lesions used in earlier studies, in the pituitary of female guinea pigs. By using various numbers of lesions they were able to destroy various amounts of the pituitary. Their lesions generally did not abolish mating, and they found no close relationship between the amount of tissue destroyed and the losses of mating behavior. Castrated females which received similar lesions could be brought into heat after appropriate injections of hormones. These and other studies have shown that there are hypothalamic sites whose destruction affect sex behavior but not through interfering with gonadotropic function. Some hypothalamic lesions do interfere directly with

remains since no female sex behavior survives ovariectomy. It may be that in this species either mounting does not depend to any great extent on hormonal status or that it depends on some non-gonadal hormone, perhaps the pituitary gonadotropins. There is at present no way to decide among these alternatives.

## II. NEURAL FACTORS

### 1. Peripheral Mechanisms

Within the last twenty years there has been increasing interest in the neural mechanisms which mediate sex behavior. Investigations have centered primarily about the role of the diencephalon, cerebral cortex, and amygdaloid complex. It is these which I shall be discussing, but since the final pathway in the sexual response is spinal I want first to mention the work on these more peripheral neural structures.

One part of the male mating pattern, the ejaculatory response, is mediated by spinal structures and cannot occur in their absence (38, 58). There is also some work with females which indicates that neural centers above the spinal cord are not necessary for some elements of the female mating pattern. Maes (50) observed female cats with the cord sectioned at a high caudal level and kept alive by artificial respiration He was able to produce raising of the pelvis, treading, and contralateral swinging of the tail after stimulation of the perineum His claim that these responses occur only during estrus has been challenged by Bard (7), who found them in anestrous females and even in males. But the essential point remains that many elements of the female pattern can be elicited with appropriate stimulation in the spinal female

Although there is the above evidence to indicate the importance of spinal mechanisms in the motor pattern of mating, earlier suppositions that afferent and efferent outflows of the genital regions are indispensable for mating behavior have not been supported by experimental work Root and Bard (55) found that male cats after removal of the three sacral spinal segments showed aggressive sexual behavior despite absence of any sensitivity in the genital region and despite paralysis of the hind limbs The animals could not, of course, gain intromission, but they vigorously attempted to mate nevertheless Animals with abdominal sympathectomy either alone or in conjunction with the above spinal loss still showed vigorous mating attempts Bacq (1) has found similar effects in the rat and guinea pig after abdominal sympathectomy The seminal vesicles and prostate, previously considered to have sensory functions necessary to the stimulation of the sex drive, also do not seem

No work has been done, although it would certainly be valuable, comparing the time and magnitude differences between lesions producing these separate effects.

3. Cerebral Cortex

The cerebral cortex has figured in a number of studies. Complete removal of the neocortex does not prevent mating responses in female rats (16, 17), guinea pigs (34), rabbits (31), or cats (6).

In the male the cortex is more important Brooks (31) reported that mating continues in the decorticate rabbit. But his data suggest that there was some deficit in these animals since removal of the olfactory bulbs, without effect when removed by themselves, completely abolished sex behavior in his decorticate males. In the rat, the only species in which quantitative destruction has been investigated, Beach (10) found that removal of 20% of the neocortex had no appreciable effects on the per cent of males mating. Destruction of 60 to 75% of the cortex abolished the mating behavior of males completely. With lesions between these limits, the drop in the percentage of males mating was approximately proportional to the size of the lesion. The locus of the lesion was less important than its size This well known "mass action" effect is similar to what has been observed in maze learning (48) and maternal behavior (8) in rats

An interesting verification of these results has been found in female rats which show male mounting responses (16). In eleven such females the cortex was first removed from one side of the brain and later removed from the other side. After unilateral removal the incidence of the male mounting responses decreased while there was no important change in the incidence of the female pattern. After complete decortication the male behavior completely disappeared while the elements of the female pattern increased slightly in frequency The effects of cortical removal upon masculine reactions thus seem to be the same whether the operation is performed on males or females

Recently Beach *et al* have completed three studies on the effects of decortication on the behavior of male cats These studies were originated and carried out at the American Museum of Natural History. In the first paper (27) mating behavior of unilaterally decorticated male cats was reported. In two of five cats, the remaining cortex was later removed. All the cats with unilateral removal continued to copulate after operation Four gained intromission on all postoperative tests This finding is quite in contrast to earlier data on the rat (10) where unilateral removal pro-

gonadotropic functions (35, 42, 43), but the behavioral effects of these lesions have not been well studied.

Hypothalamic lesions are also effective in reducing mating behavior in the male rat (32) and guinea pig (29). A recent study carried out in the Yale laboratories by Dr. Charles Rogers (54) has explored the hypothalamic mechanisms of mating in the male rat. Rogers placed electrolytic lesions, 1 mm. in diameter, at six sites in the hypothalamus. This work was done on 72 experimental animals and 60 controls. In the control animals electrodes were placed but no lesions were made.

In the experimental animals, decrements in mating behavior were associated with lesions placed in two of the six sites. Animals with lesions in the premammillary area showed a decrease in behavior which gonadal hormones were effective in restoring. Animals with lesions in the tuberal region showed behavior decrements which could not be reversed by gonadal hormones. Histological verification of these lesions has not been completed, but this demonstration of a twofold control of mating behavior in the hypothalamus supports findings mentioned above. Rogers (personal communication) has suggested three ways in which hypothalamic lesions may influence sex behavior: (1) by interference with anterior pituitary function, (2) by interference with autonomic centers in the hypothalamus, and (3) by interference with the locus of hormone action in the central nervous system. Little is known at present of the last mechanism.

Hillarp *et al* (44) have recently reported that certain lesions in the hypothalamus of rats stimulated rather than reduced mating behavior. Lesions, placed in the basal preoptic area, produced a short-lived sexual aggressiveness in both male and female rats It was characterized by vigorous mounting responses in a typical male fashion. This exaggerated sexuality appeared about 20 to 30 minutes after the animals had recovered from anesthesia It persisted for several hours and then weakened and disappeared. During this period none of the experimental females showed lordosis when mounted by males. As the authors recognized, these effects might have been due to irritation of adjacent neural structures or to the destruction of an area which normally inhibits mating.

From these studies it appears that there are tracts or nuclei in the anterior ventral hypothalamus which are essential to the execution of mating patterns in male and female rodents Some lesions produce this effect by interfering with the gonadotropic function of the anterior pituitary. In other cases the lesions disrupt sex behavior although every indication is of normal gonadotropic and gonadal hormone function.

temporal and parietal lobes had no important effects on mating In studies with the rat, motor disability was a relatively small part of the behavior loss, since once mating was initiated it was generally accomplished in an adequate manner. These differences between the effects of approximately similar lesions in cats and laboratory rats suggest an interesting species difference in the mechanisms of sex behavior.

Bearing indirectly on the role of the cerebral cortex is some work we reported recently on the effects of electroconvulsive shock on mating (22). We used a group of fourteen male rats and tested them in a control series and then exposed them to daily electroconvulsive shocks for 12 days They were tested 22 hours after the 4th, 8th, and 12th shocks Testing was continued through the following month to study recovery from electroconvulsive shock In the tests during shock treatment the animals took significantly fewer intromissions to reach ejaculation. They also took less time to ejaculate, not only because of the fewer intromissions, but also because they were bunching them closer together. On the other hand, the males took longer to initiate mating at the beginning of the test and a longer time to resume mating once ejaculation had occurred. One striking finding was that males ejaculated in 100% of the tests in which they gained intromission, whereas in the control tests they ejaculated in 61% of the tests in which they gained intromission.

During the posttreatment tests the animals continued to ejaculate in less time than before treatment. The time to initiate mating and to resume mating after ejaculation decreased so that it was within the range of the pretreatment scores.

We have interpreted these results in terms of two processes which seem to take place during mating, an arousal process and a copulatory-ejaculatory process The results suggest that electroconvulsive shock inhibits the arousal mechanism, since the animals took a longer time to commence mating, while facilitating the copulatory-ejaculatory mechanism This hypothesis has been further elaborated by Beach (20).

## 5. The Inferior Temporal Lobes

One area of the cortex which has come to assume considerable interest lately because of some rather striking findings which are associated with lesions there is the inferior temporal lobe area. Kluver and Bucy (46, 47) reported certain remarkable behavior in monkeys in which the temporal lobes had been removed. In addition to virtual loss of emotional reactions and indications of agnosia for visual objects, the animals

duced a marked drop in the number of males which mounted. After complete decortication the two cats so treated failed to mount in any of the tests. Androgen supplementation to one of these animals did not improve its behavior. In the other animal histological study of the testes failed to reveal any evidence of malfunctioning of the hormonal system

In the second study (28) lesions were placed in the frontal lobes of six cats. Two were previously unoperated, two had previously sustained injury to both occipital lobes, and two had sustained loss of the complete cortex on one side of the brain The results showed that the amount of behavior loss after frontal lobe lesions is highly dependent on what other areas have sustained lesions. Animals with frontal lobe lesions alone were not so seriously affected as animals with additional lesions elsewhere In general, the frontal lesions appeared to affect the motor abilities of the animals and not their sexual excitability. These animals were obviously interested in the females during the tests: they followed the females, they often uttered the "sex call," and they attempted a large number of mounts although they gained intromission in only a small proportion of them.

A third study (69) reported the effects of bilateral lesions to the cortex in the temporal, parietal, and occipital lobes. In two of these animals previous lesions were present In the previously unoperated animals the lesions had little effect on mating. When there was considerable invasion of the occipital areas, the male had difficulty finding the female. In one animal which was previously hemidecorticated occipital removal completely eliminated mating reactions. This was, however, primarily a visual defect, since the animal was quite able to mount and gain intromission on the two times when it was placed in actual contact with the female. In one other male with frontal and occipital lesions on one side and complete removal of the cortex on the other, mating reactions were permanently eliminated.

Certain generalizations seem warranted from these studies Sex drive, interestingly enough, did not seem to be depressed in these male cats except after very extensive lesions. In this respect the results differ from the results of comparable studies in the rat (10). In the rat destruction of the cerebral cortex seems to interfere with the general facilitating effect of the cortex on mating behavior. In the cats there was generally no lack of interest in the female, a common finding in brain-damaged rats. Second, certain lesions in the cats, primarily in the frontal areas, interfered with the motor pattern of mating. Much of the behavior decrement in these studies was due to motor disability. Lesions in the

## III. SUMMARY

I have attempted to outline in this paper some of the salient information we have on the neuroendocrine mechanisms of sex behavior. It is clear that in all the lower mammals studied the so-called sex hormones are essential for the initiation and maintenance of the biologically adequate pattern of mating We have a fairly clear idea that the lower spinal cord, the hypothalamus, the amygdaloid complex, and the cerebral cortex, in a more general way, are important structures for the sexual act

We have, however, little information on the relationship between hormone action and neural functioning. Bard pointed out in 1940 that we should like to find differences in neurophysiology between females which are in heat and females which are not (7). Except for some work with the rat (17a) and the recent work on the temporal lobes not much more is known about the neural-hormonal interaction than was known in 1940. With the recently developed techniques of studying in intact animals the effects of hormones and electrical stimulation applied directly within the cerebral mass it probably will no longer be necessary to derive most of our information from lesion and castration studies. If, with these new techniques, it becomes possible to facilitate sex behavior experimentally, investigations into the neuroendocrine mechanisms of mating will take on added flexibility, and the opportunities for elucidating the hormonal-neural interaction will be immeasurably increased

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showed distinct increases in the frequency and intensity of sexual responses. When alone the animals exhibited considerably more sex play than normal and when paired with other animals, whether male or female, they mounted and showed thrusting behavior.

Schreiner and Kling (56, 57) reported similar effects in the cat. After bilateral injury to the amygdaloid structures male cats showed a greatly increased tendency to mount estrous females. They would also mount a number of usually inadequate stimulus partners, such as agoutis, small rabbits, dogs, and chickens. Associated with this state of what Schreiner and Kling called "hypersexuality" they found that males were easier to handle and more difficult to anger. Females similarly operated also showed signs of hypersexuality, but rather than becoming docile during this period, the females tended to become more irritable than usual

Schreiner and Kling (57) found that they could reverse the hypersexuality in their males by castration and could reinduce it with injections of testosterone propionate. This is interesting because it demonstrates an interaction between neural and hormonal effects long suspected, but not previously observed. They interpreted the hypersexuality as a release phenomenon in which the normal inhibiting effects of the temporal structures over subcortical mechanisms were destroyed by amygdalectomy.

Among the dogs which have been tested in our series at Yale, lesions were placed in the pyriform cortex in two male beagles. They had been studied for two years under control conditions and they were studied for 5 months after operation The pyriform cortex was removed in two operations, approximately 10 days apart.

In contrast to the striking behavior seen in the male cat and monkey, there were no indications of a general improvement in sex behavior in these two dogs. There was a highly reliable increase in the speed with which these dogs mounted the female at the start of each test. This speed was faster than that which had been observed in any of the previous series of tests with these dogs, but most other measures were not reliably affected They were tested up to 5 months after operation, a period which includes the time in which Schreiner and Kling observed their effects. Since these authors have not presented information on the copulatory performance of their cats beyond the descriptive data, one cannot say whether the effects which they found were more general than those found in the dog.

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DISCUSSION

R. HOSKINS. In our studies of vigor, using the revolving-cage method, we attempted to increase the activity of elderly male rats by the administration of various hormones Testosterone propionate proved to be ineffective but under the influence of estrogen the activity was strikingly increased. I wonder if you saw anything comparable in the use of heterologous hormones in your studies?

A. C GOLDSTEIN By and large the finding has been that androgens have been more effective than estrogens Did you test mating activity?

R. HOSKINS No That was not done

A C GOLDSTEIN The general finding has been fairly consistent that androgens have produced the most marked effects in males

W C YOUNG Dr Hoskins' question prompts me to say something about the problem of hormone specificity. We are questioning the opinions with respect to specificity which are found in the literature Actually, Dr Goldstein, you and Frank Beech are relatively conservative For example, Eayrs at Birmingham goes so far as to say there is no truly specific relationship between any one hormone and the pattern of behavior it facilitates. Kinsey, Pomeroy, Martin, and Gebhard state in their book on the sexual behavior of the human female that the designation "sex hormones" is unfortunate because it is only one of the agents which steps up metabolism, including the nervous function, and therefore sexual activity

I have recently reread the literature fairly carefully As you have pointed out, heterologous sexual behavior is often seen in animals receiving hormones of

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of male and female rhesus monkeys, showing that once there has developed antipathy between a pair, as, for example, after he has beaten her up, there is absolutely nothing more doing between them.

CHAIRMAN R W GERARD. There speaks a married man.

A. C GOLDSTEIN In some of the other work on dog experimentation, although our number of observations is very small we have very clear evidence of that and it does not even have to be such a specific act as biting the male that will keep them off There is one observation, a female consistently fought off one male but readily accepted the mating advances of another animal

C G HARTMAN On the other hand, there are monkeys that "vibrate" in unison, as it were I recal one female of which a certain male was particularly fond So long as he could see her out in the paddock he would not copulate with any other female presented to him When we moved the female, he became normally promiscuous again

Also may I ask you Do you feel that learning has a lot to do with subsequent behavior? And one more point, then I will finish: In the opossum, after ovulation, the corpus luteum develops with unusual rapidity and marked pseudopregnant phenomena ensue within 24 hours If in that short period I [illegible]led to separate

*their own sex*, and *the treatment* of individuals with heterologous hormone frequently is followed by the display of *sexual behavior characteristic of their own* sex However, and these words are taken from many of the reports, the responses are often difficult to elicit, displayed at a very low level, slow and weak, rudimentary and incomplete.

There are a number of other points. In the laboratory mammals that all of us have used nothing is known of the fate of the hormones we have been injecting. Particularly when large quantities of certain heterologous hormones are injected, isn't it possible that some of the hormone normally secreted by the recipient is a product of the metabolism of the heterologous hormone and could be exerting *some effect*? This may not be as probable as the suggestion that *there is an overlapping* which would *account for some of the heterologous* behavior.

Most important to me are the instances of specificity now on record There is the synergistic action of estrogen and progesterone in the female to which you have referred The action of progesterone in that case is quite specific. *It was shown by Hertz, Meyer, and Spielman a good many years ago that seven steroids* they substituted for progesterone were without effect Recently Burns and Shipley have substituted adrenosteroids for progesterone in estrogen-conditioned guinea pigs They found that those adrenal steroids were only one-fourth to one-fortieth as effective

Now coming to the *running activity which Dr Hoskins mentioned* Estradiol benzoate is almost ineffective in stimulating running activity in the rat. Estrone, on the other hand, is completely effective, not only in the female, but also in the male.

R. Hoskins *Stilbestrol* does *the same thing*

W C Young Recently, Harold Antliff compared the effects of testosterone propionate, estrone, and α-estradiol benzoate on the restoration of patterns of behavior in the male guinea pig He found that estrone is almost as effective as testosterone but that α-estradiol benzoate is not effective at all *All of this has led us to this point of view that there is quite a high degree of specificity.* However, there isn't the dichotomy, male hormones and female hormones Rather, there is a chemical specificity as in the instances of running activity, and as in the case of the estrogens administered to the male Finally, I will suggest for investigations in this area that a procedure be employed which *has not often been employed* in studies of specificity. The pattern of behavior of individual animals should be determined prior to gonadectomy. The test of specificity is the restoration of that particular pattern by replacement therapy

A. C. Goldstein I would like to make one point *Do you* believe that the individual patterns *of response are completely* washed *out in using* mean scores? Is this the import of your last statement?

W. C Young. They could be to some extent if the mean score is used, but not if reliance is placed on the qualitative record from which the score is calculated.

C. G. Hartman: *You have in the dog, domesticated so long, "intelligence"* correlated with a convoluted brain Have you noticed any cases of personal antipathy between the male and the female? It seems to me that this is a matter of great importance. It may be even more significant in monkeys *I have* records

of male and female rhesus monkeys, showing that once there has developed antipathy between a pair, as, for example, after he has beaten her up, there is absolutely nothing more doing between them

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Also may I ask you: Do you feel that learning has a lot to do with subsequent behavior? And one more point, then I will finish. In the opossum, after ovulation, the corpus luteum develops with unusual rapidity and marked pseudopregnant phenomena ensue within 24 hours If in that short period I failed to separate the pair, the female often killed the male, even though he was several times her size. Why he would submit to the beating was always a mystery to me. I have looked through quite a number of books on two points, the neural mechanism of (1) erection and (2) ejaculation I could not find the complete answer even in such a good textbook of physiology as that of Bard, himself an authority on related mechanisms I gathered that the prevailing opinion is that the sacral sympathetic conditions erection, the sympathetic the act of ejaculation It seems to me that we need researches in this area

A. C. GOLDSTEIN There is one of these questions to which I wish to reply. That is on the role of learning I can furnish information on the nature of the learning factor on the effects of dogs' learning patterns that will get them into a mating situation. But with reference to the experiment that I did mention, the only information we have on the effect of experience is that there is no clear-cut prediction from the number of tests that these dogs had before castration in relation to the mating pattern that they showed after castration, so on the limited experiments the effects are not clear, but this does not mean that no such effects are present

B B BRODIE The workers in this particular area of research are not as enterprising as the psychiatrists in coining new terms, otherwise they would be using the term erospharmacology

I would like to bring out a curious difference in the effect of the sex hormones. Female rats when given barbiturates sleep about five times as long as do males. This difference reflects the rapidity with which the male rate metabolizes barbiturates compared to the female. Going into the problem further, it was shown that the enzyme systems in liver microsomes that metabolize barbiturates are much more active in the male rats than in the female. After the male rat is given estradiol for a few days he will react to barbiturates like a female, and correspondingly the activity of the detoxication system in microsomes will have decreased On the other hand, if the female rat is given testosterone, she will soon react to barbiturates like a male, and correspondingly the activity of the detoxication mechanism in

microsomes will have increased. Curiously enough, the rat is the only laboratory animal that shows this sex difference to barbiturates, and is the only species whose reaction to barbiturates can be changed by sex hormones It makes one wonder if there may be other examples where conclusions from the effects of hormones on one species can not be extrapolated to other species

E. ANDERSON. In regard to the removal of the amygdala, you mentioned that the effect in dogs is not like that described in cats by Schreiner and Kling Our observations in male dogs are similar to yours, namely that there is no evidence of increased sexual activity after bilateral removal of the amygdala Dr. Wilson in our laboratory, has studied the pattern of urinary steroids before and after the bilateral amygdalectomy in these male dogs. No change was found in either the fractions containing androgen metabolites (17-ketosteroids) or in the corticosteroid fractions Have you tried making more extensive lesions in the same area?

A. C. GOLDSTEIN: No, ours were fairly restricted lesions.

E. ANDERSON: I was interested in your observations on dogs with lesions in the preoptic area which showed an increased response. We found the adrenal steroid excretion very much increased in dogs with lesions in the preoptic region. The response was equivalent to a dose of 150 mg. of ACTH

A. C GOLDSTEIN: The dogs which showed the increased response had lesions in the pyriform area Increased response after preoptic lesions was found in rats (44).

G. PINCUS: I would like to make a remark about the suggestion of metabolic effect. I think it very unlikely that estrogens would be metabolized to any of the steroids with more than 19 carbons. All attempts to find this out by the use of tracer estrogens are negative You will not get androgens as far as we can see from work in animals and man. Androgen, however, is convertible to estrogen and this has been demonstrated both *in vivo* and *in vitro* In fact, the *in vivo* observations date way back to observations of Koch who saw increased estrogen excretion after the administration of androgens. There is likely to be conversion, but the extent of that conversion, at least on the basis of our present knowledge, may not be very large On the other hand, the amounts necessary for the reflexes in which you are interested may be pretty small The specificity of estrogen which Dr. Young mentioned is very fascinating Actually as far as we know, in the case of all animal and human studies there tends to be an equilibrium between estradiol and estrone One would expect a certain amount of estradiol to be converted to estrone The fact that estrone is very specific indicates that the amounts of estradiol which are metabolized to estrone cannot be very high.

CHAIRMAN R W GERARD I think there is time for one more question, and I would like to ask it I have been interested to note the large amount of discussion on specificity as concerning the hormones involved and the very little discussion of specificity as concerning the neural regions involved It seems worth while to pick up Young's point that, if one merely changed the total level of activity of various parts of the nervous system, one would not expect to change those patterns of behavior which can be influenced by taking out one neural region or another, that is, getting release phenomena or stimulation phenomena It has always seemed to me fairly obvious that these must depend upon rather specific biochemical regional differences, down to cellular differences Two points may be worthy of mention on

that. First, I don't recall the detailed experimental evidence, but the kind of experiments Harris was doing with fetal animals led him to conclude that, since one could transplant male or female sex glands or pituitaries, without altering the genetically expected sex, the primary sex difference was in the hypothalamus and that the sexually specified hypothalamus controlled the endocrine sex differentiation.

Second, in picking up the point that Brodie made, one could make a considerable advance in a direct attack on the action of sex hormones on the male and female rat brain by looking for enzyme changes in microsomes of specific brain regions That is, if you could show that some change was rather uniquely limited to the hypothalamus, reticular formation, or wherever it may be, it would be a strong point in the direction you indicate. Do you care to add anything on that?

B. B BRODIE: It is an interesting idea

C. G. HARTMAN. The following observation, it seems to me, illustrates the fact that a behavioral pattern resides in the nervous system: A normal bitch is never seen to raise her hind leg in urination, but I seem to recall some experiments where she will use the city fire plug after the manner of a male after she has been treated with testosterone.

# SEROTONIN, EPINEPHRINE, AND THEIR METABOLITES IN RELATION TO EXPERIMENTAL PSYCHIATRY

# Serotonin in Mental Disorders

D. W. Woolley

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Confronted with the task of presenting evidence which indicates that serotonin is concerned in mental processes, I feel much as Charles Darwin must have when he was asked to present in 20 minutes the evidence for his view of the origin of species. Just as in Darwin's case there was a body of evidence, no single piece of which was above question, so also then is the situation with respect to serotonin. The limitation of time will necessarily make it seem that the support for the serotonin idea is very meager whereas it would be much more impressive if it could be given in detail.

To state the case briefly, I should say that during the past two years evidence has been accumulated which indicates that the hormone serotonin has a function in maintaining normal mental processes, and that interference with its action in the brain leads to mental disorders and neurological dysfunction. I wanted to talk about the experimental basis of this idea today. This concept was first enunciated by Woolley and Shaw early in 1954 (16, 17). It was based on their studies of antimetabolites of serotonin, the first of which was published in 1952 (13). Since that time a variety of observations made by other investigators, as well as by ourselves, have lent support to the original idea. Time will not permit mention of all of the evidence, nor will it even permit a description of all of that portion of it which we ourselves have been able to assemble. Consequently, I propose to mention only certain findings which, in the present state of understanding of the matter, seem to be of particular relevance.

First, let me say that no proof exists that serotonin controls normal mental processes. What does exist is a body of evidence which indicates that tampering with the functioning of this hormone results in behavioral and psychiatric changes in man and in laboratory animals. These changes frequently resemble those seen in naturally occurring mental diseases. In addition, drugs such as Reserpine and chlorpromazine, which are said to be of some benefit to certain patients suffering from mental diseases, have been shown to influence the action of serotonin in suitable pharmacological tests.

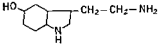
Because the idea that serotonin might be concerned in mental processes arose from studies of antimetabolites of it, let us first refresh our memories about what an antimetabolite is, and how it functions. In any living thing there are a number of chemical compounds which are specifically essential for various vital processes. These are called essential metabolites, or, more simply, just metabolites Examples are vitamins such as thiamine, ascorbic acid, etc., and hormones such as thyroxine, testosterone, serotonin, adrenaline, etc. Each of these metabolites serves as a specific substrate for a special metabolic reaction. The substrate fits the enzyme of such a reaction just as a key fits its lock, and the turning of the lock opens the door of some special living process. We may picture serotonin as one of these keys which fits a lock called the serotonin receptor. When the two interact we see the result usually as the contraction of a smooth muscle, because it is this contraction of smooth muscles caused by serotonin, which is the most studied manifestation of its action

An antimetabolite of serotonin is a chemical compound which is shaped to resemble this hormone Because of this structural resemblance, the antimetabolite can be thrust into the serotonin receptor, i e., the special lock, just as the hormone itself can be However, because the antimetabolite differs in some detail from the real metabolite, the lock cannot be turned by it. It therefore occupies the space and excludes the normal entry of serotonin. The result is the creation of a specific deficiency of this hormone. We see this effect of the antimetabolite in an animal (or in one of its isolated tissues) by a failure to respond to administered serotonin. Such an animal still responds normally to other metabolites just as locks are not blocked by keys unrelated to the proper ones for them

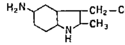
This crude mechanical analogy permits us to visualize what an antimetabolite is, and how it functions. An antimetabolite of serotonin is thus a structural analog of it, and one which is able to exclude the hormone itself from its receptor thereby creating a deficiency of it. The structure of serotonin is shown in Fig. 1 The first two antimetabolites of it, which were synthesized in 1952, are also shown there

Dr. Shaw and I had started the search for antimetabolites of serotonin in the winter of 1951-1952 in the hope of thereby discovering a drug useful in the treatment of essential hypertension (13) Some synthetic compounds, including those shown in Fig 1, were produced and found to act on smooth muscles as antagonists to the contracting action of serotonin Before this work had proceeded very far it was realized that

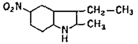
various naturally occurring drugs were structural analogs of this newly discovered hormone. It was then quite easy to show that these drugs actually did function as competitive antagonists of serotonin on smooth muscles. This made it seem quite probable that part at least of their pharmacological action was due to this antiserotonin property. The drugs were actually naturally occurring antimetabolites of serotonin (7, 15).



Serotonin

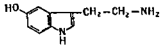


Amino analog

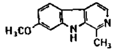


Nitro analog

FIG. 1

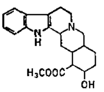


*Serotonin*

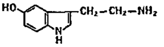


Harmine

FIG 2



Yohimbine

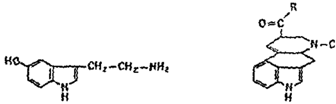


Serotonin

FIG 3

There were three principal classes of such drugs These were the harmala alkaloids of which harmine (Fig. 2) is an example, yohimbine, and *its more recently discovered congener, reserpine (Fig 3), and the* ergot alkaloids such as ergotamine and lysergic acid diethylamide, or LSD (Fig 4). The arrangement of the figures in this order makes evident the gradually increasing molecular complexity. The structural resemblance of harmine to serotonin is quite plain, but without having seen the intermediate steps, you might want to argue that yohimbine and LSD are not analogs of it. In our studies of 1952-1953 each of these

substances was shown to act as a competitive and reversible antagonist of serotonin when tested on segments of carotid artery or rat uterus. A few data to illustrate this point for LSD are shown in Table I, and for harmine as tested on the rat uterus, in Fig. 5. Much more extensive data on these and related compounds can be found in the original papers and in subsequent publications from the laboratories of Dr. Gaddum (5) and of others.



Serotonin

Ergot alkaloid
(When R is diethylamino,
= Lysergic acid diethylamide,
When R is a peptide,
= Ergotamine, Ergotoxine, etc)

FIG 4

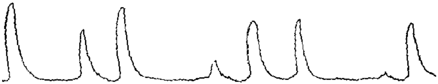



FIG 5 Response of rat uterus to serotonin and to harmine
S = serotonin
W = wash
H = harmine

Several of these naturally occurring antimetabolites of serotonin were known to cause psychiatric disorders in men and to cause behavioral changes in animals Thus, LSD especially had been found to be outstanding in this respect because of the minute doses of it which would call forth a psychiatric state in man resembling schizophrenia (10). However, it is well to remember that a prominent feature of ergotism in man was the mental or psychiatric changes which were reviewed long ago by Barger (1), and to recall that since there is no LSD in natural ergot, the other ergot alkaloids must be capable of causing these effects if the dose is large enough. Similarly harmine has long been known to

cause a type of deranged behavior in dogs and other animals. The aboriginal use of crude yohimbine as an aphrodisiac may be taken as evidence that this compound, as well, affects the mind.

These psychiatric effects of compounds which we had just found to be antimetabolites of serotonin were engaging our attention when we were fortunate to find that some of the new, synthetic antimetabolites of serotonin were capable of causing behavioral changes in animals. The nitroindole shown in Fig. 1 had been the first promising antiserotonin suitable for trial in hypertension (14). When it was studied for toxicity in mice prior to human trial, I noticed that a few of these animals which

TABLE 1

SEROTONIN VERSUS LYSERGIC ACID DIETHYLAMIDE (LSD) IN THE CONTRACTION OF CAROTID ARTERY RINGS

| Serotonin (μg per cc.) | LSD (μg per cc.) | Decrease in diameter of ring (per cent) |
|---|---|---|
| 0 | 0 | 0 |
| 0.2 | 0 | 31 |
| 0.2 | 0.03 | 23 |
| 0.2 | 0.1 | 15 |
| 0.2 | 0.3 | 12 |
| 0.2 | 1.0 | 6 |
| 0 | 10.0 | — 3 |

had eaten large amounts of it for a long time showed a change in character. They became savage and would run toward me and bite my hand when I fed them. This was an ill-defined change and was not seen in all the mice, and was consequently of questionable significance. When this same compound was given to Dr. R. Wilkins for clinical test, however, the significance of the mouse result was plain. This antimetabolite of serotonin caused profound mental depression in humans.

At about this same time, a new antiserotonin was synthesized and found to cause convulsive fits in mice. This was medmain, the structure of which is shown in Fig. 6. This was a potent antiserotonin when tested on smooth muscles (8), but when it was given to mice in rather large doses it caused convulsive fits which were impressively reminiscent of epileptic seizures in man.

Since that time, we have made other close structural analogs of serotonin which have called forth in dogs irrational behavior. Other laboratories also, in their search for antiserotonins, have had similar ex-

periences. Thus, a few analogs of serotonin, which have shown no striking toxicity in laboratory animals have been given clinical trial in the treatment of hypertension, but have had to be abandoned because of psychiatric side effects. It was not until we had fully appreciated the danger of causing undesirable effects on the mind, and had found a way of getting around these effects, that it was possible to produce a compound which showed clinical promise in hypertension. Such a compound has now been produced (19). We may view the finding of mental disorders caused by certain antiserotonins as a byproduct of these efforts against hypertension.

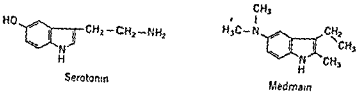



FIG 6

The reason why serotonin got into psychiatry, then, was because of pharmacological studies with smooth muscles. Several of the analogs of serotonin, which acted as antimetabolites of it, caused psychiatric changes in men and behavioral changes in animals This correlation was first made in 1954 (16, 17) and it was argued that since the effect of the antimetabolites on muscle was to cause a deficiency of serotonin, the mental effects of the same compounds might be the manifestation of a similar serotonin deficiency somewhere in the brain

Although this idea of deficiency of serotonin is a plausible one, there is another view of the data which must be taken into account This is that the analogs of serotonin which affect the mind do so because they act like serotonin rather than antagonistically to it This alternative was considered in the original paper (17) and it has grown a little in stature since. To return to the lock and key analogy one would say that some of the serotonin relatives fit well enough into the serotonin receptor of the brain to be able to take the place of the hormone and carry out some of its functions One might also picture them as acting to inhibit the enzyme (amineoxidase) in the brain which normally destroys excess serotonin. Inhibition of this enzyme would lead to the piling up of excess serotonin. Because this hormone is a substrate for this enzyme, a structural analog might be expected to inhibit it. Two modes of action, both leading to an excess of serotonin in the brain might thus be pictured, and

are alternatives to the idea that a deficiency of cerebral serotonin is the cause of the psychiatric disorder. To distinguish with certainty between these two alternatives is very difficult. The need to do so is great because it is vital to the understanding of the disease and to rational attempts to control it via the serotonin route. Let me illustrate the nature of the dilemma.

Although LSD has been shown by many workers in a variety of pharmacological tests to be a potent and a specific antagonist of serotonin, it can also be shown to act like serotonin Marrazzi and Hart were the first to find evidence of this in the optic cortex. Recently we also have found that just as serotonin stimulates the heart of the clam *Venus mercenaria,* so also LSD stimulates it. Getting closer to home phylogenetically, we have also observed that just as serotonin caused rises and falls in the arterial blood pressure of a dog, so also LSD caused these same effects In fact, on a weight basis, LSD was about three times as powerful as serotonin in both of these tests. Furthermore, the rise in blood pressure of the dog which was caused by LSD was prevented by a specific antimetabolite of serotonin (Fig. 7). This was additional evidence that the action was serotonin-like. The antimetabolite used in this experiment was the one recently found to be active in human hypertension without causing mental disorders (19). Its structure is shown in Fig 8 Clearly then, LSD has some serotonin-like actions.

Not only does LSD have serotonin-like as well as anti-serotonin properties, serotonin itself will act as an antiserotonin and will do so quite specifically. Gaddum showed that an excess of the hormone would render the rat uterus insensitive to the amounts which ordinarily would cause it to contract (4). Shaw and Woolley (9) have shown this same sort of antiserotonin action in living dogs in the blood pressure test. This type of effect is readily understood in terms of the lock and key model. The serotonin receptor is simply blocked by several molecules of the hormone all attached to the site at which one molecule must combine if the hormone is to exert its usual effect. This point should not be mystifying because it is the well-known phenomenon of inhibition of an enzyme by an excess of its substrate

We must now ask ourselves whether all of the other analogs of serotonin which have been found to affect the mind and the central nervous system likewise have a serotonin-like action If they do, then the idea of serotonin excess rather than deficiency as the causative factor must be given prominence. When harmine was tested on isolated rat uterus for serotonin-like action none was found (Shaw and Woolley, unpublished

data.) Similarly, when harmine was tested for pressor action in dogs, none was found. However, in the blood pressure test, subsequent challenge with serotonin revealed an enhancement of its action in the case of the dog, and an antagonism in the case of the rat uterus. Similarly, the nitroindole shown in Fig. 1 was only an antagonist of serotonin in the dog, but Welsh (11) found it to act like serotonin on the heart of the

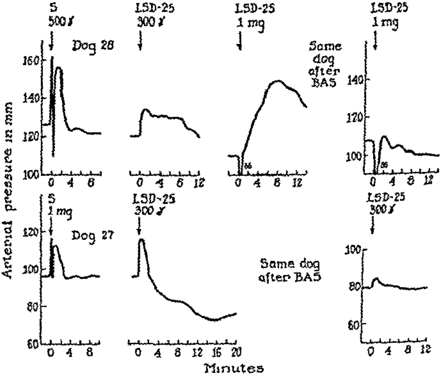
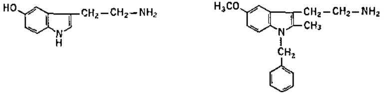



FIG 7 50 mg of BAS given i v one hour before last LSD-25 injection

clam. Medmain was found by Shaw and Woolley to be only an anti-serotonin on sheep arteries, but to have some serotonin-like action on isolated rat uterus (8). All of this indicates clearly that whether one finds antiserotonin or serotonin-like effects very often depends on the tissue or species chosen for study. It is useless to attempt to decide the matter by finding an hallucinogenic compound which acts only as an anti-serotonin on all tissues The risk is always great that although on ten test objects a compound will act only as an antagonist, on the eleventh it may show some serotonin-like effect. Several substances have been found to act like serotonin and to be at the same time and on the same

*tissue antagonists of it Serotonin itself* as described above is a case in point. I believe that you will appreciate that this point of an excess or a deficiency as the causative factor is not easy to resolve. Especially is this true when attempts are made to infer from isolated muscles which of the opposing actions is being exerted on human brain

You will ask why this question of too much or too little serotonin has not been solved simply by injection of this hormone The reason is that in animals, peripheral injection leads to no increase in the brain content. In other words, peripherally administered serotonin passes into the brain only in amounts too small to measure. Direct injection into the brain has been tested in animals, but is not something which one can do in human




Serotonin

1 Benzyl 2,5 dimethyl serotonin
Benzyl analog of serotonin (BAS)

FIG 8

beings The chief impression gained from animals given intracerebral serotonin is lethargic behavior, a drowsiness and unwillingness to move normally. If the dose is increased to gigantic amounts (1–5 mg per kg.) this lethargic state may give place to violent convulsions, resembling those provoked by medmain. The natural precursor of serotonin, namely 5-hydroxytryptophane, has been shown by Udenfriend and his collaborators to pass the blood-brain barrier, and to increase the serotonin content of the brain Peripheral injection of this precursor in huge amounts does lead to convulsions in some species and we have seen it cause marked central effects in dogs and mice It will be of much interest to learn whether it is hallucinogenic in man. However, it must be pointed out that neither intracerebral serotonin nor 5-hydroxytryptophane will cause mice to behave in the fashion they do when given LSD If the presumed psychotic behavior of these animals is to be attributed to its serotonin-like action, we must ask why serotonin will not reproduce the effect. This behavioral test will be described later in this discussion.

Thus far the demonstrations of antiserotonin action have been made on isolated smooth muscle preparations, or in animals in which one

measures a peripheral effect such as change in blood pressure You will want to know whether there is any justification for transferral of such results to arguments about action in the brain. Indeed you will want to know if serotonin has any demonstrable action at all on the brain We also have been troubled by these questions, and have made the following experiments in an effort to study them Through the cooperation of Doctors M. Murray and H Benitez of Columbia University it has been possible to find a visible effect of serotonin on a special kind of brain cell cultured *in vitro*. Furthermore, it has been possible to show that a few of the antimetabolites of serotonin will overcome this action on the brain cells just as they antagonize serotonin in smooth muscles

In the brain there is a special kind of cell, the oligodendroglia, which have a pulsating movement. This contraction and expansion of these cells reminded us of the characteristics of smooth muscles on which serotonin acts It seemed possible that these structures would be caused to contract with serotonin. Both human and rat oligodendroglia were examined in tissue culture, and it was found that serotonin did in fact cause them to contract strongly Dr. Murray has made a time-lapse movie which shows the normal pulsation and the effect of serotonin quite strikingly.

This effect of serotonin was not shared by other kinds of brain cells, because astrocytes were not caused to contract.

The contractile effect of serotonin on human oligodendroglia was overcome by synthetic antimetabolites of serotonin such as medmain and 1-methylmedmain Application of these compounds in low concentration to the contracted cells caused the resumption of normal pulsation. The antimetabolites caused no discernible effect at this same concentration when applied to normally pulsating cells in the absence of serotonin. The closeness of correspondence between the results obtained on smooth muscles (rat uterus) and those on the brain cells was striking indeed. Especially was this true with respect to one curious difference between medmain and 1-methylmedmain. These two antimetabolites of serotonin are very closely related in chemical structure, and they have equal potency as antiserotonins when tested on rat uterus as well as when tested on human oligodendroglia However, at higher concentrations, medmain, but not 1-methylmedmain, has a serotonin-like action on uterus It was of much interest to see that the same was true on the oligodendroglia. What was even more interesting was that medmain, but not 1-methylmedmain, caused the convulsive fits in mice We had earlier associated this convulsant action of medmain with its serotonin-like effect, and had

regarded it as a serotonin-like compound which, in contrast to serotonin itself, was able to penetrate into the brain readily. The ability to demonstrate all of these findings again in brain cell cultures just as they had been found to exist in smooth muscle preparations, encouraged us to believe that the results on smooth muscles have a bearing on the reactions of brain to these compounds.

The action of LSD also was studied in human oligodendroglia. The results were more complex than with the two medmains The first effect seen with LSD was a relaxation and vacuolization of the brain cells. This was then followed by a strong, serotonin-like contraction. Under suitable conditions of concentration one could prevent the initial flaccidity by administration of serotonin. In this respect then, an antagonism was demonstrated. The subsequent contraction was not antagonized by serotonin, in fact, it was augmented by it In this system then, LSD showed both pro- and antiserotonin actions just as is the case in the test which uses blood pressure responses of dogs.

These studies with oligodendroglia are only beginning, and much remains to be done They seem to show that one can find a visible action of serotonin on the brain, and they may provide a means of testing which is much more relevant than are assays with smooth muscles. The reader is referred to the original paper for sequential photographs which illustrate the effects which have just been briefly summarized (2).

The demonstrable action of serotonin on oligodendroglia provides us with a clue about one of the possible ways in which interference with serotonin in the brain may lead to hallucinations and convulsions. This is probably not the only function of this hormone in the brain and much more will need to be done to prove the following idea. Nevertheless, it may have some value since it seems to account for many of the phenomena which have thus far been seen. Because the brain is poorly vascularized in comparison to organs such as kidney, it has seemed that some means is necessary to insure adequate circulation of extravascular fluids in order that oxygen, glucose, and other requisites reach the cells rapidly enough. The pulsations of the oligodendroglia, and their anatomical situation in the brain, suggests that these cells are little stirring devices which circulate the extravascular fluid If they were to be stopped, either by a tetanic contraction or by flaccidity, then this circulation would be impeded It is well known that convulsions are a common result of anoxia of the brain It is not so well known, but nevertheless true, that hallucinations and most terrifying anxiety can be evoked in a normal man just before he loses consciousness in hypoglycemia. I my-

self have experienced this effect called forth by injection of insulin. Anoxia and hypoglycemia of the brain are thus known to elicit the effects which we wish to connect with serotonin. However, it must be stated again that there may be other more direct actions of serotonin on neural [illegible] ibly one of many. It is [illegible] the brain to ascribe to serotonin we are a long way from the psychiatric problem. For this reason we have sought to devise tests of the ability of serotonin and its relatives to influence measurable mental processes. The only adequate test is one involving human beings because they are the only ones in which mental illness can be diagnosed. We have therefore been pleased to see that two Italian psychiatrists, Montanari and Tonini (6) have quite recently stated that the mental effects caused by LSD have been overcome in men by parenteral injection of serotonin. I hope that this finding will be substantiated in other laboratories. However, some time before the appearance of this clinical report, similar evidence had been found in studies with mice in our own laboratory (12). The attempt to devise tests based on animal behavior was made because we have no access to patients or to human experimentation, and because many types of experiments cannot be done on human beings.

In devising such tests one must remember that a mere neurological finding of abnormal behavior might tell us very little. Several poisonous agents elicit neurological abnormalities. For this reason behavioral changes have been sought which allow one to interpret the change in terms of a mental derangement. Nevertheless, it is a dangerous extrapolation the limitations of which one must never forget.

The following test was devised. Mice given relatively large doses of LSD followed a train of behavior which was regular and easily reproducible. They became agitated, looking rapidly from side to side, they sank low to the ground, and spread their forelegs and pushed backwards actively. Soon they began to walk backwards. This type of behavior was observed in normal mice placed on an inclined plane of glass. Just before the plane was raised to such an angle that the animals began to slide downhill they exhibited this kind of behavior. This suggested that the LSD created in the mouse the sensation that it was about to fall or slide downhill.

This presumed hallucination, or at least the behavioral change, could be prevented by administration of serotonin and a cholinergic drug directly into the brain. Peripheral administration of these agents did not

prevent the LSD-induced backward walking. Furthermore, the protection was not 100 per cent as can be seen from Table II.

This demonstration might be considered as evidence that the LSD had induced an hallucination in the mouse by creation of a cerebral deficiency of serotonin, and that injection of the serotonin had made up for this deficiency and thereby prevented the mental change However, this would still leave unexplained the need for the cholinergic drug. Possible explanations of this need have been discussed elsewhere (12). Furthermore, the dose or serotonin was large in comparison to the total amount in a mouse brain This might suggest that serotonin was being

TABLE II

ANTAGONISM BETWEEN LSD-25 AND SEROTONIN PLUS CHOLINERGIC DRUGS IN THE ABNORMAL BEHAVIOR OF MICE[a]

| I.C Injection | No of mice | No pushing but not walking backward | No walking backwards |
|---|---|---|---|
| Saline | 19 | 0 | 19 |
| Serotonin 20 μg | 5 | 0 | 5 |
| Carbamylcholine 0 2 μg | 13 | 0 | 13 |
| Serotonin 20 μg + Carbamylcholine 0 2 μg | 23 | 3 | 14 |
| Physostigmine 1 0 μg | 5 | 1 | 4 |
| Serotonin 20 μg + Physostigmine 1 0 μg | 5 | 3 | 2 |

[a] All mice given 100 μg of LSD-25 each intraperitoneally

rapidly destroyed by amineoxidase, but other explanations may apply. Taken at face value, the experiments support the idea of a serotonin deficiency as the cause of the presumed mental change. The Italian findings referred to above in human beings would offer confirmation of this view.

Recently Cerletti and Rothlin (3) have reported that brom-LSD, a very close relative of LSD, and one which like LSD acts as an antiserotonin on smooth muscles, will not cause hallucinations in men. They have put this observation forward as proof that the original suggestion of Woolley and Shaw is untenable I believe that the idea is not so readily disposed of Several antimetabolites of serotonin have been known not to cause behavioral changes in animals even though some do. This aspect of the problem has been discussed in the original paper of Woolley and Shaw, and subsequently (18) and need not concern us here. What

is of interest is that the brom-LSD even in very large doses has failed to cause mice to walk backwards. This would suggest that the mouse behavior test correlates in this instance with the psychiatric findings in men.

To summarize, I feel that sufficient evidence has been found to suggest that serotonin plays a role in the brain, and that pharmacological interference with this function there may influence mental and neurological processes. There is no proof that these relatives of serotonin do not affect other processes aside from those concerned with serotonin, and these other processes may be of great importance However, the use of analogs of serotonin has brought to light so many phenomena related to mental function as to suggest a participation of this hormone in normal mental processes.

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### Discussion

CHAIRMAN R. W GERARD *Thank you very much, Dr Woolley Like Darwin, you have marshaled a great deal of evidence, the next few minutes will indicate whether you have been as convincing.*

S S KETY If Dr Woolley had confined his presentation to his last few sentences, I would agree wholeheartedly I think it an extremely provocative thought that serotonin may have something to do with mental processes That however is somewhat different from an attempt at the present time to give a more

definite concept of exactly what role it does play in mental processes Therefore, since Dr Woolley has proposed at least one definite theory, he expects, I am sure, that we will be quite free in indicating our reservations regarding it I should like to point out that one may retreat from the evidence on smooth muscle, that is the antagonism exhibited by LSD and other drugs to the smooth muscle effects of serotonin, as not being precisely relevant to the mental effects of these drugs Indeed, one is almost forced to retreat in the light of the evidenc of Rothlin and Dr Woolley himself, who have found many serotonin antagonists which have no mental effects But in retreating one must realize that the smooth muscle antagonism of LSD and serotonin formed the most important basis for the hypotheses relating serotonin to psychosis in the beginning

D. W Woolley· May I speak on that before you go to another point? First let me dispel the illusion that the original evidence was based on LSD It was not It was based on harmine, yohimbine, LSD, and synthetic antimetabolites If you will read my original paper you will see that all of these were involved The evidence did not rest on LSD alone.

With regard to Cerletti and Rothlin, they have demonstrated that brom-LSD will not cause hallucinations in man but acts similarly to LSD on smooth muscle My original paper was published in 1954 If you will go back and read that paper you will find that we discussed that very point, since we had antagonists of serotonin at the time that did not produce mental phenomena So we did take this into account in our original proposal

In regard to the mouse-behavior test, we have tried some brom-LSD. This will not make mice walk backwards as LSD does We think that the mouse test is of some value It is a mistake to say that the original idea arose from LSD. That was only one of the points of evidence

S S Kety I did not mean that the original idea arose from LSD In the first slide you showed that the demonstration of antagonism, be it with LSD or with medmain or other antimetabolites, rested on smooth muscle assays Therefore it does not seem exactly consistent now to insist that the smooth muscle is not an important analog of the mental effects, a thesis to which I would have agreed originally

D W Woolley I hope I did not leave the impression that I thought that I was retreating as you said from the smooth muscle findings I am not retreating at all I am trying to extend the observations beyond smooth muscle We still use smooth muscles most actively We think the results obtained with them mean a great deal, but we think the gap beween smooth muscle and the brain may be a very large one and many people such as yourself will be wary of jumping it

S S Kety The studies on the effects of serotonin on the oligodendroglia are certainly interesting There is no doubt that we don't understand how many substances get into the tissues of the brain

It is extremely difficult to explain how radioactive phosphate, for example, injected into the ventricle will get into the brain in a matter of 30 minutes when by the simple process of diffusion it can be shown that it would take many hours for this to occur and, therefore, the idea that the oligodendroglia, whose function is somewhat mysterious anyhow, serve as a sort of pumping mechanism is extremely attractive However, there are certain questions that one might raise. In the

first place, it is my understanding that these contractions and relaxations are very slow and are made rapid only by the tremendous acceleration of the motion pictures that have been taken. But more important than that, oxygen and carbon dioxide can diffuse apparently quite freely through all of the membranes in the brain There does not seem to be any blood-brain barrier for these gases and one can show on the basis of diffusion theory, despite the fact that the brain is not as vascular as the heart, that it is, nevertheless, quite vascular enough to account for the oxygen requirements and the carbon dioxide output of the brain entirely in terms of their diffusion to and from the nearest vascular structure.

More important than that, if this mechanism were correct and as a result of a paralysis there were a suffocation of the brain in LSD psychosis, then one would expect to find some changes in the metabolic rate of the brain in terms of oxygen, or carbon dioxide, or glucose under the effect of LSD or some evidence for a tissue anoxia or a piling up of carbon dioxide Dr. Sokolof and others of us at NIH, have made such measurements in human beings, who were actively hallucinating and found that there were no changes in $CO_2$ output, oxygen, or glucose consumption in the brain as a whole. Admittedly one could say that this mechanism and its paralysis were highly localized, but it seems that that again is altering the hypothesis in the face of evidence which seems to suggest that perhaps another hypothesis would be a more heuristic one.

CHAIRMAN R. W. GERARD. Before you answer, I want to say one thing, on that same point, which has been in my mind ever since I first heard you suggest this in New York. I made a quick estimate from the movie of the rate at which these oligodendroglia cells were pulsating, when you reduced it from the time lapse photography. As I remember, it was about one beat in 20–30 minutes

D. W. WOOLLEY: No, systole three minutes, diastole 2 minutes This agreed with the original finding of Pomerat

CHAIRMAN R. W. GERARD. This is not the speed of the observations shown on the film

D. W. WOOLLEY. Dr Murray may have made a slip of the tongue If you look at the paper on the subject you find that it is stated there

CHAIRMAN R. W. GERARD Even five minutes is an awfully slow beat to do any significant micro pumping and I completely agree with Dr. Kety on his reaction to that particular mechanism

D. W. WOOLLEY Let me say one word first, Dr Kety Please don't forget what I said, to wit. I think this is one mechanism which we cannot fail to heed but it is not the only mechanism I myself don't think this is the only one.

*Here is the situation In the brain one has a poorly vascularized tissue, between* the capillaries and the neurons are the oligodendroglia. To deny that they are stirring up the fluid in which they are resting is denying the fact The only question of the significance of this is how much of a role it plays in the pharmacologic events We just have to wait till we get more data to see but I think we cannot afford to neglect this in looking for other possible ways in which these compounds act

H. HOAGLAND. I would like to ask if your hypothesis of the lock and key analogy would fit the type of data that both Marrazzi in his laboratory, and Slocombe in mine, have obtained in studies of electrical activity in the brain in

which LSD and serotonin together with adrenochrome, adrenaline, noradrenaline, all have the same type of action For example, under conditions of anesthesia with pentothal all these substances decrease the frequency of the electrical activity recorded from both cortex and depth electrodes Serotonin is the most potent. It is effective in doses of 1 μg. in slowing the electrical activity This slowing is characteristic if one uses pentothal anesthesia If one uses ether or has the animal not anesthetized—I am speaking of the work of Slocombe with rats—one does not see the slowing Facilitation is more characteristic

We have also recorded the transcallosal response in rats that Marrazzi has described in cats These substances all have inhibitory action in the pentothalized rat and so we have confirmed Marrazzi's finding in the cat Would you, Dr Woolley, interpret this in terms of your analogy by saying that in these cases these substances all turn the key in the lock and show no competitive action for receptors because they all fit the lock so well? Would this be interpretable in terms of antimetabolite action where the effects of the drugs are in the same direction and show no competition

D W Woolley May I say that the lock and key analogy is a very simple one on which to hang our thinking, so that we understand each other. One cannot push this analogy too far My own thinking based on evidence that others have obtained, as well as that which we have ourselves found, is that there is more than one kind of serotonin receptor in an animal I think Gaddum has produced evidence to show that in the small intestine, for example, he can distinguish two kinds of receptors With this complexity before us we must be very careful in our thinking and in our experimentation. However, I think the lock and key analogy is a good one, as a starting point on which to base our thinking It is also somewhat more than an hypothesis. Thus, for example, it has been demonstrated that many other antimetabolites work by actually excluding, pushing out the related metabolite Along this line we have been able to show that some of our synthetic antimetabolites of serotonin displace serotonin from the receptors

Dr Brodie, likewise about a year ago, showed that reserpine which we considered to be an antimetabolite of serotonin, also does this very effectively. Other antimetabolites of serotonin can do it too This is not surprising because this is a way a number of antimetabolites have been shown to work They displace the metabolites from the locks, you might say, and the metabolites are then not available

H Hoagland You would not feel that the similarities of effects of this group of drugs on the brain, rather than competitive actions which some of them show on smooth muscle, modify the lock and key analogy?

D W Woolley I don't feel it modifies it at all I think the picture of the lock and key fits thus far adequately enough to enable us to use it Thus inhibition caused by the excess of serotonin, the antiserotonin effect of excess serotonin, as well as excess LSD and all the other things is an exact duplicate of the inhibition of ATPase by large amounts of ATP There are any number of enzymatic models upon which you could call to illustrate the same point

A S Marrazzi As a matter of fact, this discussion has saved me a lot of time on what I wanted to say We approached the problem from a different point of view, using a different technique, namely, by studying the evoked poten-

tials in the cat cortex to determine synaptic effects In this way we demonstrated the synaptic excitatory action of cholinergic agents and inhibitory action of adrenergic agents. We postulated some disturbance of the chemical synaptic regulation by certain psychotogens because of the structural similarity of the LSD and mescaline to serotonin and adrenaline and we did find that they produce synaptic inhibition As a hypothesis we thought this might underlie the mental disturbance produced. Serotonin was the most active of all in producing cerebral synaptic inhibition This, I think, adds to Dr. Woolley's thesis. Furthermore, there seems to be some pertinence of these animal experiments to the clinical situation because clinically these psychotogens rank in potency in the same order as they do as synaptic inhibitors in the cat Again clinically the tranquilizers all are effective in varying degrees All offset the effects of mescaline and LSD In our experience the tranquilizers do offset the cerebral synaptic inhibiting effect of mescaline We have not as yet adequately tried them against serotonin.

I was very happy to note Dr. Woolley's elaboration of the receptor concept to explain apparently that in this instance the peripheral receptors are not as good indicators of central action as they might have been, although he now has brought them into a formulation that would be acceptable on the basis of competition of similar substance for a similar receptor

S. Udenfriend. Since we have heard this thing on glial cells, I wonder whether we could get full details on that. For instance, I don't know much about how much LSD was used, the concentrations, or the amounts of serotonin that had to be added *in vitro* We said something about the time lapse We were not told anything about specificity I know there have been papers on it, but I don't recall what epinephrine or histamine does Could we have some of those data?

D W Woolley. In the manuscript which I prepared for this occasion and which I did not read, I said these experiments are in the beginning We started this work about a year ago last November As to the amount of substances and our procedure with the glial cells, explants are made from human embryonic brain or from embryonic rat brain. These are grown on slides with serum The slide is dipped into a solution of serotonin, 5 μg per cubic centimeter, dipped in and taken out and observed under the microscope It takes around 30 minutes to get maximal contraction, which is a long tetanic one, lasting from 2 to 3 hours before there is relaxation Then the cells gradually return to normal pulsation Now with respect to the amount of antagonist, with medmain the inhibition index was identical with that found on rat uterus It takes ten times as much medmain as of serotonin to get complete antagonism With LSD the amounts were varied For purposes of discussion let us talk about 5 μg per cubic centimeter. In the first 30 minutes one sees flaccidity, failure of contraction, vacuolization of the cell After about an hour strong tetanic contraction, such as you get with serotonin, sets in, and it lasts for around 2 hours Now how much do we use when we reverse the LSD action? We apply 5 μg LSD, and 2 μg of serotonin at the same time, 2 μg of serotonin alone will not do anything to these cells However, 2 μg. of serotonin will prevent the vacuolization and flaccidity of the cells treated with LSD To indicate the specificity of this effect, astrocytes treated in the same way with serotonin do not contract

S UDENFRIEND What about histamine and other cells?

D. W. WOOLLEY: We have not done anything with histamine.

A G SLOCOMBE What is the latency of the contraction of the glial cells with 2 μg of serotonin and without the LSD?

D. W. WOOLLEY: We do not have exact measurement of latency, but the 2 μg. of serotonin is insufficient to cause the tetanic contraction of the cells. We hoped to be able to find that with the amounts of serotonin which are insufficient to cause these tetanic contractions, one could demonstrate the action against LSD Whether the concentration of serotonin one finds in the brain would be able to increase the period of systole has not yet been determined

B B BRODIE. In considering Dr Woolley's hypothesis, it should be remembered that the concentration of serotonin in brain averages about 0 5 μg per gram of tissue, virtually all of which is bound I understand that in your experiments, the concentration of serotonin, in a free form, was about 5 μg per milliliter of solution

D W WOOLLEY Yes, 5 μg serotonin per cubic centimeter is more than the concentration in the brain, but I would remind you that the contractions produced by this quantity are very profound It is quite possible that smaller amounts, such as those found in the brain, will increase the period of systole or diminish the diastole

B B BRODIE One finding I do not understand is the effects of serotonin on the uterus *in vitro* and its antagonism by LSD If we talk in terms of a lock and key, then there is no door, since the uterus normally contains no serotonin It could be argued that serotonin is being formed and destroyed with extreme rapidity in the uterus with the resultant net level of the amine too low to measure, although high enough to have physiologic activity But this seems improbable since the uterus is normally one of the most quiescent organs in the body How can we explain the presence of receptor sites for serotonin in an organ which normally contains none of the indole?

D. W WOOLLEY We have to be very cautious in saying it does not play a role I think we have to reserve our judgment on that First let me say that the uterus will not respond to serotonin except during estrus

B B BRODIE It will respond, but not so strongly

D W. WOOLLEY The order of difference is about one thousand times· in order to get a non-estrus uterus to contract you must apply between a thousand and ten thousand times the amount of serotonin It is too early to say that serotonin plays no role in the uterus

A S MARRAZZI I want to make a further comment pertinent to the amount of serotonin We have dealt of course, with the synaptic inhibition produced by serotonin, so the amount that would be important to us would be the amount of serotonin at the synapse We tried to approach that in a very preliminary fashion by adding a monoamine oxidase inhibitor and we produced exactly the serotonin effects in the animal If we can repeat that with small enough doses of iproniazid, the assumption is that there is enough natural serotonin protected at the synapse to produce the action

One more point, of course I think most of us, certainly I, would be perfectly agreeable to saying that, if this is an underlying mechanism for some sorts of

mental disturbances, it need not be serotonin but may be something having those properties which would be produced by a perversion of metabolism.

D. W. WOOLLEY: There is no proof that the substance which occurs in the brain is not bufotenin.

B. B. BRODIE: There is no measurable bufotenin in the brain.

D. W. WOOLLEY: What are the measurements?

S. UDENFRIEND: The paper is in press. Essentially all the 5-hydroxyindoleamine in tissues is serotonin. No bufotenin was detected.

I cannot resist a nostalgic comment. I am so vividly reminded of a decade or so ago when another substance started to become exciting. I wonder how soon we can school ourselves to remember that when any agent is experimented with extensively a great many actions of it are likely to turn up, so that only an intellectual moron cannot traverse a theoretical path from one or another of them to almost any conclusion he wishes to reach. The problem is, of course, what the quantitative relations are and the magnitudes of changes under different conditions. I say this without any prejudice pro or con serotonin, but it is a point of view that I do think has to be kept in mind by all of us, when a tremendous amount of data starts to pour in as a result of finding some substance with a lot of interesting actions.

# Biochemical Studies on Serotonin and Their Physiological Implications

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Although in this discourse serotonin will be considered solely from the standpoint of neurochemistry, it must be pointed out that the brain is only one of the tissues in which serotonin is found. In fact, of the serotonin in the body, the amount in brain, represents but a fraction of a per cent, the largest amount being found in the gastrointestinal tract where its function is yet to be explained It is present in the blood, and has recently been found in lung (20). Serotonin is found in many tissues of almost all mammals and invertebrates investigated, it is also found in toad venom (15) and in the octopus salivary gland (7) 5-Hydroxyindole compounds have even been found in plants (13, 22) and in bacteria (9) where they serve as intermediates in the formation of pigments. We must, therefore, consider the 5-hydroxyindole pathway of tryptophan metabolism an important one With its widespread occurrence in nature there probably will be many important functions ascribed to it

This paper will first describe some of the analytical techniques that have been developed for serotonin and its metabolites. Then it will present biochemical studies concerning the identification of serotonin, its localization in the central nervous system, localization of the enzymes involved in its synthesis and metabolism, intermediates of metabolism, and the nature of the enzymes involved. From these studies we will then turn to those aspects which might be of physiologic or pharmacologic interest, enzyme inhibitors and their *in vivo* effects, the effects of deficiencies of some of the coenzymes involved in serotonin metabolism, and the effects of the serotonin precursor, 5-hydroxytryptophan, on experimental animals

The bioassay techniques, used by Twarog and Page (14) and by Amin *et al.* (1) to identify serotonin in brain do not distinguish between serotonin and related substance such as the methylated analogs Spectrophotofluorometric studies in conjunction with countercurrent distribution

and bioassay have now confirmed the presence of serotonin in brain and have ruled out the occurrence of methylated analogs (3).

In strongly acid solution serotonin and other 5-hydroxyindole compounds exhibit a characteristic fluorescence; when activated at 295 mμ they fluoresce at 550 mμ (17). In pure solution, one can readily detect 0.02 μg of serotonin per milliliter. Brain homogenates are extracted from an alkaline pH into butanol and reextracted from butanol into acid (3). In the course of these extractions substances like 5-hydroxyindoleacetic acid and 5-hydroxytryptophan are removed, only basic substances being extracted. The material extracted from brain in this manner, when subjected to a nine-plate countercurrent distribution, distributed itself in the same manner as authentic serotonin. The distribution coefficient of bufotenin in this system is so different from that of serotonin that if it were present it would have been almost entirely concentrated in the first plate whereas serotonin would be found in plates four and five. Actually less than 1% of 5-hydroxyindole fluorescing material in the brain extracts appeared in the first plate. The material extracted from brain and subjected to countercurrent distribution was assayed both fluorometrically and by the clam heart bioassay. The values obtained by both methods were found to agree and the first plate did not contain any material which could activate the clam heart. These experiments make it fairly certain that the 5-hydroxyindoleamine in brain is serotonin and not a methylated analog

The second problem concerns the distribution of serotonin in the brain. Is it localized in specific areas or is it randomly distributed? Studies on cat and dog brain indicate that it is found in highest concentration in the hypothalamus and more primitive centers, little being present in the cerebellum and cortex (2). Gaddum and Giarman (8) have reported similar findings. This pattern of distribution may be a clue to the role of serotonin in brain.

The next problem deals with how serotonin gets into the brain Is it made there or is it brought there from peripheral sources? Apparently serotonin penetrates into the brain with great difficulty. If one administers relatively large amounts of serotonin it is, first of all, very rapidly metabolized. Even if one maintains a relatively high blood level in dogs and rats there is no detectable rise in the brain serotonin level. However, with huge amounts of serotonin administered to mice, it has been reported that one can get a perceptible increase in the brain (10). Apparently traces can cross the brain barrier but the huge amounts administered peripherally in order to accomplish this are hardly physiologi-

cal. Further evidence that serotonin is not made parenterally and transported across the brain comes from studies on patients with malignant carcinoid (see below), a tumor which makes huge amounts of serotonin (12). The blood level in such patients can be 10–20 times normal and the urinary excretion of 5HIAA elevated over 100 times normal. With this huge amount of serotonin being synthesized peripherally one cannot detect a trace of serotonin in the spinal fluid. Furthermore, patients with malignant carcinoid show no demonstrable central disturbance.

The most convincing evidence that serotonin is made within the brain, of course, is the demonstration of enzymes involved in its synthesis. In animals and in patients it has been demonstrated that tryptophan when administered in radioactive form yields serotonin (18) The intermediate in this conversion is 5-hydroxytryptophan (5HTP). This amino acid has only recently been isolated from the urine of a patient with malignant carcinoid (6). In animal tissues there is an enzyme that decarboxylates 5-hydroxytryptophan to serotonin (16). It is present in huge quantities in most tissues and is highly specific for 5-hydroxy-L-tryptophan. It does not act on tryptophan. As far as the metabolism of serotonin is concerned a major part of parenterally administered serotonin can be isolated as 5-hydroxyindoleacetic acid (5HIAA) in the urine (16). The enzyme monoamine oxidase is responsible for the oxidative deamination (11) leading to 5HIAA.

Methylation of serotonin occurs in the toad, in invertebrates, and also in plants The finding of traces of dimethyl serotonin (bufotenine) in human urine has also been reported (4). However, in this laboratory no such compound has been detected Furthermore, attempts to demonstrate methylation of serotonin by animal tissues *in vitro* have not been successful.

Thus far little is known about tryptophan hydroxylase in animals and in man. However, it is hoped that studies in bacteria and toads may yield clues which will be of help in studies on the mammalian enzyme.

5-Hydroxytryptophan decarboxylase is found in relatively high concentration in the brain It, too, is not uniformly distributed in this organ, in general the localization of this enzyme parallels that of serotonin Gaddum and Giarman have reported findings of a similar nature (8). Some of the properties of this decarboxylase are of interest and can be useful in further studies on the role of serotonin First of all, the enzyme, like all amino acid decarboxylases, requires pyridoxal phosphate (5, 21) which is derived from vitamin $B_6$ Pyridoxine deficiency is associated

with neurological disturbances. It was of interest, therefore, to find out what happens to the serotonin depots and to the enzyme, 5HTP decarboxylase, in animals deficient in this vitamin. The animals used were 6-week-old chicks which were raised from hatching on a diet containing about 1 to 2 per cent of the optimal amount of pyridoxine. These were compared with pair fed controls, so there were no differences in their food intake They were somewhat smaller in size but the values for serotonin in Table I are given in terms of microgram per gram of tissue

TABLE I
TISSUE LEVELS OF SEROTONIN IN NORMAL AND PYRIDOXIN-DEFICIENT CHICKENS[a]

| Tissue | Serotonin (μg/gram) |
|---|---|
| Brain (Normal) | 1.13 (7) |
| Brain (Deficient) | 0 39 (4) |
| Intestine (Normal) | 5 00 (5) |
| Intestine (Deficient) | 1.19 (6) |
| Blood (Normal) | 2 90 (5) |
| Blood (Deficient) | 0 60 (5) |
| Liver (Normal) | 1 60 (5) |
| Liver (Deficient) | 0 42 (5) |

[a] Chickens were fed from hatching on a diet containing about 1% of the optimal quantities of pyridoxine. Their food consumption was similar to that of the controls. The values shown for each experiment are averages, the numbers in parentheses indicate the number of animals employed

It is apparent that the amount of serotonin in the various depots, including brain, is markedly diminished in pyridoxine deficiency. This effect of pyridoxine deficiency is interesting but it is probably not specific for serotonin. Since pyridoxine is involved in all amino acid decarboxylations it should also be involved in the formation of epinephrine and norepinephrine, and in the formation of histamine. If pyridoxine deficiency influences epinephrine, norepinephrine, and histamine as much as it does *serotonin it is indeed not surprising to find such marked neurological* disturbances.

The next aspect of the decarboxylase concerns studies with 5HTP *in vivo.* Would it give rise to intracellular serotonin? If it did what would be the consequences? It has been found that when 5HTP is administered to animals it is taken up by most tissues and there converted to serotonin (19). Thus almost all tissues, even those which normally contain no appreciable amounts of serotonin, become rich in serotonin. Increased amounts of serotonin are found in hypothalamus, cortex, and other parts

of the brain When one administers 5HTP and measures both the amino acid and serotonin in various tissues, one finds that the amino acid enters into tissues such as brain and there is converted to serotonin. Elevated brain serotonin levels are obtained following the administration of 5-hydroxytryptophan Serotonin itself, when administered as such, does not end up in the brain (see above). 5-Hydroxytryptophan, like other amino acids, can cross the blood-brain barrier and there be converted to serotonin 5-Hydroxytryptophan thus provides a parenteral method for getting serotonin into the brain

When dogs were given 60 mg of 5HTP per kilo, intravenously, they showed marked central disturbance. The following effects were seen: skeletal tremors, loss of placing reactions, postural incoordination, lacrimation, salivation, piloerection, increased heart rate, marked gastro-intestinal activity, loss of response to visual stimuli, pupillary dilation and loss of the light reflex. These effects are similar to those produced by the hallucinagenic indole, lysergic acid diethyl amide. We have here a situation where the brain serotonin level has been increased enormously, the levels in the dog, at this dose, being at least ten times the normal level One has to attain levels several times normal to obtain such effects

What brings about these effects? When 5HTP is administered it enters the brain and also gives rise to serotonin and 5-hydroxyindoleacetic acid. Any one of these substances could therefore be responsible for the observed effects By administering 5HTP with an amine oxidase inhibitor isopropyl isonicotinyl hydrazide (iproniazid), it has been possible to show that the effects are related to serotonin alone. With a given dose of 5HTP the amounts of brain serotonin found in animals pretreated with iproniazid are much greater The effects of 5HTP are also intensified. However, little change is seen in the amounts of the amino acid in brain. When 5HTP was given to rats in doses of 100 mg per kilogram the brain serotonin level rose from 0 6 to 1 3 $\mu$g per gram No central effects were observed at this dose However, when the animals were pretreated with iproniazid the serotonin level was increased to over 4 $\mu$g per gram At these levels there were observed the same effects as are produced by L.S D in these animals. The iproniazid produced little effect on the brain level of 5HTP. Unless they are pretreated with iproniazid, rats and mice require enormous amounts of 5HTP to show central effects. On the other hand, cats, dogs, and rabbits show these effects without such pre-treatment. Actualy 5HTP raises the amount of serotonin not only in the brain but in all portions of the body Caution will have to be observed, therefore, in determining which of its effects are central and which peripheral.

The metabolism of serotonin is apparently catalyzed by the enzyme monoamine oxidase which is present in large amounts in brain (11). Zeller (23) had been studying this enzyme for many years before its relationship to serotonin became evident. However, much remains to be learned about this enzyme. Is there one monoamine oxidase or are there several? How can it be solubilized and isolated from the mitochondria? What are its properties and cofactors? We know of many substances that can inhibit monoamine oxidase *in vitro* Iproniazid, as was shown above, can also be useful *in vivo*.

A comparison of the distribution of 5HTP decarboxylase, serotonin, and monamine oxidase in various parts of the brain is shown in Table II. In general, areas that are rich in serotonin are also rich in the decarboxylase. Monoamine oxidase is found in large amounts throughout the brain although there appears to be a little more in the hypothalamus than in other areas. It is evident that potentially more serotonin can be destroyed than can be made in a given time It must be concluded therefore that serotonin, as it occurs, is undoubtedly bound or in some way separated from monoamine oxidase.

Now what happens when one inhibits the enzyme monoamine oxidase *in vivo*? When iproniazid is administered to rats or rabbits, in doses of over 50 mg per kilo, within a very short time there is observed a large increase in brain serotonin. Within 5 hours, the increase is about threefold. Apparently one can produce an increase in brain serotonin by administering an inhibitor of monoamine oxidase. If one were to assume that inhibition was complete then the rate of formation of serotonin in the brain must be relatively rapid, the half-life being of the order of an hour or two Radioactive studies on serotonin turnover in the gut indicate that its half-life there is at least 10–12 hours. Serotonin in the brain is apparently made more rapidly

Although iproniazid can produce demonstrable changes in serotonin in brain it is not a very efficient inhibitor If mice are pretreated with iproniazid the rate of serotonin metabolism by the whole animal is unaffected. However, homogenates of tissues from the same animals were found to be unable to destroy serotonin When intact tissue slices from these animals were compared with the homogenates it was found that the monoamine oxidase activity of slices was not inhibited whereas that of homogenates prepared from the same tissue was totally inhibited. Thus conclusions concerning *in vivo* action based on studies with homogenates are misleading and although iproniazid has demonstrable anti monoamine oxidase activity *in vivo* it is not nearly as effective as

data from homogenates would indicate. In mice, pretreatment with iproniazid produces a large increase in the amount of serotonin appearing in brain after a given dose of 5HTP but the effects on serotonin in the carcass are hardly significant.

TABLE II
THE DISTRIBUTION OF SEROTONIN, MONOAMINE OXIDASE, AND 5HTP DECARBOXYLASE IN DOG BRAIN

| Tissue | 5HT content in µg/g | 5HTP decarboxylase activity expressed as µg 5HT formed per gram per hour | Monoamine oxidase activity expressed as µg 5HT destroyed per gram per hour |
|---|---|---|---|
| Amygdala | 2.1 | 17.6 | 968 |
| Hypothalamus | 1.5 | 117 | 1624 |
| Hypothalamus | 1.8 | | |
| Septal Area | 1.5 | 109 | 1217 |
| Midbrain | 1.0 | 97.6 | 842 |
| Pyriform cortex | 0.94 | 15.5 | 926 |
| Caudate nucleus | 0.55 | 306 | 935 |
| Caudate nucleus | 0.98 | | |
| Medulla | 0.64 | 32 | 1117 |
| Thalamus | 0.50 | 38 | 940 |
| Thalamus | 0.64 | | |
| Hippocampus | 0.64 | 15.5 | 1176 |
| Olfactory bulb | 0.39 | — | 573 |
| Pons | 0.38 | 28 | 936 |
| Cortical gray matter | 0.27[a] | 7.2 | 819 |
| Cortex | 0.17[b] | — | 844 |
| Cerebellum | 0.09 | 9 | 930 |
| Corpus callosum + internal capsule | —[c] | 4 | 466 |
| Lateral geniculate | —[c] | 7.9 | 844 |
| Medial geniculate | —[c] | 7.6 | 865 |
| Fornix | —[c] | 9.6 | 707 |
| Optic tract | —[c] | 7.5 | 701 |

[a] Gray matter taken at random from the neocortex

[b] A slice of tissue containing both gray and white matter

[c] Even after combining tissues from two animals no 550 mµ fluorescence could be detected

Iproniazid is thus a useful tool. The fact that it may have selectivity for brain may make it even more valuable. However there is need of a monoamine oxidase inhibitor that is more potent than iproniazid and which can penetrate into cells better than iproniazid.

I think it is now justifiable to say that serotonin must have a role

The metabolism of serotonin is apparently catalyzed by the enzyme monoamine oxidase which is present in large amounts in brain (11). Zeller (23) had been studying this enzyme for many years before its relationship to serotonin became evident. However, much remains to be learned about this enzyme. Is there one monoamine oxidase or are there several? How can it be solubilized and isolated from the mitochondria? What are its properties and cofactors? We know of many substances that can inhibit monoamine oxidase *in vitro*. Iproniazid, as was shown above, can also be useful *in vivo*.

A comparison of the distribution of 5HTP decarboxylase, serotonin, and monamine oxidase in various parts of the brain is shown in Table II. In general, areas that are rich in serotonin are also rich in the decarboxylase. Monoamine oxidase is found in large amounts throughout the brain although there appears to be a little more in the hypothalamus than in other areas. It is evident that potentially more serotonin can be destroyed than can be made in a given time. It must be concluded therefore that serotonin, as it occurs, is undoubtedly bound or in some way separated from monoamine oxidase.

Now what happens when one inhibits the enzyme monoamine oxidase *in vivo*? When iproniazid is administered to rats or rabbits, in doses of over 50 mg per kilo, within a very short time there is observed a large increase in brain serotonin. Within 5 hours, the increase is about threefold. Apparently one can produce an increase in brain serotonin by administering an inhibitor of monoamine oxidase. If one were to assume that inhibition was complete then the rate of formation of serotonin in the brain must be relatively rapid, the half-life being of the order of an hour or two. Radioactive studies on serotonin turnover in the gut indicate that its half-life there is at least 10–12 hours. Serotonin in the brain is apparently made more rapidly.

Although iproniazid can produce demonstrable changes in serotonin in brain it is not a very efficient inhibitor If mice are pretreated with iproniazid the rate of serotonin metabolism by the whole animal is unaffected. However, homogenates of tissues from the same animals were found to be unable to destroy serotonin When intact tissue slices from these animals were compared with the homogenates it was found that the monoamine oxidase activity of slices was not inhibited whereas that of homogenates prepared from the same tissue was totally inhibited Thus conclusions concerning *in vivo* action based on studies with homogenates are misleading and although iproniazid has demonstrable anti monoamine oxidase activity *in vivo* it is not nearly as effective as

## Discussion

A. G. Slocombe: Is it not possible that because of the large amounts of serotonin that you are injecting that you may be changing the blood-brain barrier, since when we inject 0.1 µg per gram there is an immediate change of cortical activity, so something seems to get to the brain under these conditions?

B. B. Brodie: Dr. Slocombe, how did you inject the serotonin?

A. G. Slocombe: By the intracarotid route.

S. Udenfriend: We never measured the effects of serotonin injected intracarotidly. We now have radioactive serotonin and we would like to measure that. Actually what I would try to point out is that Erspamer suggested that the enterochromaffin cells in the gastrointestinal tract were the organs which made serotonin and speculated on the passage of serotonin from these tissues into other parts of the body. I would say there would be very little possibility of finding that measurable amounts pass through the circulation into the brain.

A. G. Slocombe: I have to inject ten times the amount in the femoral vein to get the same effect as in that following carotid injection. This may be due to destruction of serotonin by passage through the liver. With reference to the blood-brain barrier, don't you think it is possible that you inject so much that you may actually alter the permeability of the barrier?

S. Udenfriend: Cortical effects do not prove that serotonin gets into the brain. Vaso-constriction and anoxia can have marked effects too.

A. G. Slocombe: They can, but it does not appear to happen in these experiments. There is no vascular cortical change which can be detected under the dissecting microscope.

G. H. Glaser: What is the cortical effect you observe?

A. G. Slocombe: In pentothal anesthetized animals, we find a complete flattening of spontaneous activity without any appreciable effect on either respiration or electrocardiogram.

G. H. Glaser: The effect described by Dr. Udenfriend on dogs suggests a subcortical localization particularly in the midbrain, the tremors, the pupillary dilatation, loss of the light reflex, loss of placing reactions, and postural incoordination, suggest a disturbance in the midbrain in addition to the hypothalamic activity.

A. G. Slocombe: In the pentothal-anesthetized animal that is true.

G. H. Glaser: It is therefore interesting that cortical effects also occur.

A. G. Slocombe: We find the flattening of activity in the tegmentum, thalamus, hypothalamus and on the cortical surface in the pentothal-anesthetized but not in the ether-unanesthetized rats.

E. Anderson. Dr. Glaser mentioned the midbrain symptoms in these dogs and I should like to point out the resemblance of the midbrain dog to the picture which Dr. Udenfriend described in the dogs given 5-hydroxytryptophan. Immediately after transection of the midbrain, there is increased activity of the gastrointestinal tract, the bowels move frequently and in some cases clear mucus or blood and bile stained mucus continues to be passed for days. There is excessive salivation and increased gastric secretion. The dogs vomited readily. The animals which survive for months and finally stand and walk show difficulty in placing reactions,

in the function of the brain. Evidence for this is (a) serotonin is found in the brain, (b) the enzymes which can make serotonin and destroy it are also found there, and (c) increasing the amounts of serotonin in brain, as with 5HTP, or decreasing it by pyridoxine deficiency, produce marked central disturbance. The pharmacological effects produced by indole drugs are further proof of an important central function for serotonin.

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very considerably As Slocombe has suggested the blood-brain barrier may have become extremely resistant.

D W. WOOLLEY: One thing about the experiment that troubles me, Dr. Marrazzi, is how the serotonin gets through the blood-brain barrier. All sorts of minor trauma, cuts and bruises and the like, destroy enough platelets to liberate more than a microgram of serotonin. Can you measure such serotonin release resulting from the behavior of the animal so you can recognize its effects following various stresses, cuts, and the like, that liberate serotonin many times greater than when the cat gets a microgram of serotonin injected into the blood stream?

A S. MARRAZZI We are not talking about a microgram in the systemic blood stream, but a gamma in the fairly close arterial injection in the common carotid which feeds directly in the cortex

S S KETY. I can't accept the permeability of the blood-brain barrier to serotonin merely on the basis of nonspecific alterations in electrical activity in the brain until you gentlemen are able to convince me that these are not the effects of local constriction of blood vessels. Evarts tells me that in his experiments with close arterial injection of serotonin he got obvious blanching of the brain Admittedly, that was a higher dose than the ones that you had used, but it demonstrates that serotonin like epinephrine can in certain concentrations have a marked vasoconstrictive effect on the brain As Dr. Marrazzi knows, I do not feel that he has ruled this out even for his earlier effects with epinephrine

One must also bear in mind the question of dosage It is somewhat misleading to report dosage in terms of kilograms of body weight when the injection is directly into one carotid, since the concentration in that vascular bed would be at least 10 times and may be as much as 100 times greater than that achieved by intravenous injection, so what looks like a small dose reported one way, may actually be a vasoconstrictive dose in the affected vessels

A S MARRAZZI· As Dr Kety said, Dr Evarts' doses were relatively enormous We also looked at the brain, although looking does not tell you very much We cannot see any obvious change, and this also Slocombe has told is true in the rat Dr DiStefano in Hodges' department has tried to measure the oxygen utilization by recording the DC potentials at the time serotonin was given and he finds that the changes do not coincide in such a fashion as to indicate a circulatory or capillary bed change Unfortunately his oxygen electrode was on the symmetrical point on the opposite slab of cortex, not on the same point where the serotonin inhibition of evoked potentials was recorded However, there are other indirect pieces of evidence For example, one can get synaptic inhibition with substances that will either raise systemic blood pressure tremendously or cause it to fall, so it is not dependent upon at least the passive change in the blood flow through the brain that can be produced With mescaline the blood pressure falls, and we get a synaptic inhibition With adrenaline the blood pressure rises and we get synaptic inhibition

G S GORDAN Do the high blood levels of serotonin in the carcinoid reflect serotonin that actually gets into the carotid artery? Aren't those venous levels?

S UDENFRIEND We measured and found serotonin in many vascular beds, arterial and venous In fact, in occasional patients we have found a relatively high free serotonin level, serotonin not bound to platelets

they stand with front legs crossed and hind legs spread apart. Vision seems to be limited to distinguishing between light and darkness. The pupils are widely dilated, even in presence of a bright light When the animal is restrained, it goes into a state suggesting rage with struggling, biting, hyperventilation and profuse salivation

CHAIRMAN I. H. PAGE: How did you prepare the dog?

E ANDERSON. Our dogs have a high midbrain transection One dog has survived almost a year.

A. S. MARRAZZI: What did you do? Did you inject something?

E. ANDERSON. No, but the picture Dr. Udenfriend described when he gave 5-hydroxytryptophan resembles the dogs with midbrain transection

S. UDENFRIEND: You probably know that malignant carcinoid patients have hyperserotonemia. I doubt whether you would detect any difference in their brain serotonin levels.

A. G. SLOCOMBE: Might this not also be due to alteration of the blood-brain barrier?

S. UDENFRIEND: I do not know, but one can find carcinoid patients that have all gradations of serotonin from large amounts to small.

B. B BRODIE: It is probable that giving serotonin by femoral vein or by the carotid artery constitutes two quite different experiments If relatively large amounts of serotonin are given intravenously, the peripheral effects will be prominent, but little central action may be evident, since most of the indole may be metabolized or localized in tissues, especially platelets, before it reaches the brain On the other hand, the intracarotid injection of an amount of serotonin, too small to have a marked peripheral action, may induce a central action because it passes through the brain in a free form.

A. S MARRAZZI: It seems we are sort of skirting the issue. We are really not defining it and this is the difference between chemical measurements and physiological ones. It is perfectly true that when we put in serotonin intracarotidly we get profound effects, inhibition of the evoked cortical response This is in keeping with the very considerable knowledge that minute quantities of substances can have profound effects on the central nervous system An example of this sort of thing is magnesium narcosis, where no one has yet found any increase of magnesium in the brain during magnesium narcosis It is pretty evident that this must be a central effect I don't think any real argument exists that serotonin gets through the blood-brain barrier with relative difficulty, but it can get through This business of the clinical effects of high blood levels could be very easily passed off, at least for the sake of argument, on the basis of adaptation slowly developing with gradually increasing concentration in the blood Furthermore, Dr. Udenfriend, did you not say at one time that one of your patients did have symptoms?

S. UDENFRIEND Only one of about eighteen carcinoid patients showed signs of central disturbance

A S. MARRAZZI: So what I am trying to say is that there is no real argument. It may do so with difficulty, but it really gets in

R. W. GERARD. The criteria of carcinoid are also physiological

A. S. MARRAZZI. There is a situation where adaptation has changed the picture

chosis, but there is an interesting clinical account of pyridoxine deficiency in children quoted some time ago, in which infants were fed one of these standard preparations that unfortunately lacked pyridoxine These children developed convulsions The cause of the convulsions was not known until the formula was worked out and it was found that it was pyridoxine-deficient Pyridoxine was added, the deficiency was relieved and the convulsions no longer appeared. This has led to a study of spontaneous pyridoxine deficiency which is rather rare It may be one of the causes of unexplained convulsions

G Pincus I would like to ask Dr Udenfriend a question about the state of serotonin He is talking of bound serotonin and free serotonin, and I would like to ask first, what is the bound state in any of the tissues that he studied, and secondly, is there any difference in the activity of the bound serotonin and free serotonin?

S Udenfriend That can be easily demonstrated One of the tests for serotonin, biologically, uses the small intestine, the best source being guinea pig ileum You can get marked sensitivity with small amounts of serotonin, yet the ileum contains lots of serotonin present in much higher concentration than you add to the bath We can reproduce this situation if we give 5-hydroxytryptophan to an estrus rat We can then obtain uteri with concentrations of serotonin up to 2 to 5 μg per gram These uteri are still sensitive to 0 01 μg of serotonin as are uteri of animals without serotonin This would mean that if you have several grams of uterus you can have a thousand times as much in the uterus and still have the same sensitivity to added serotonin This serotonin is not washed out The same is true of serotonin in platelets and I think Dr Brodie has something to say about the evidence for release of it from the platelets into the blood

A S Marrazzi I would like to ask about the data of Dr Udenfriend on brain serotonin The caudate nuclei, the hypothalamic segment, the pallidum, and the midbrain show similar values, but there are very wide decarboxylase variations and the oxidase variations are also very wide and not in the same direction What does this mean? What is the significance of these enzyme concentrations?

S Udenfriend I did indicate that the amygdala was an anomaly However, the decarboxylase and serotonin concentrations are in pretty good agreement, indicating that where it is made you find a lot of serotonin (Table II)

The oxidase is present everywhere in huge amounts and may indicate that any small amounts of amine that leak out from where it is bound may be toxic and must be rapidly destroyed

A S Marrazzi My point is that perhaps these concentrations are not such sensitive indicators once you have an adequate amount You may have a great excess which may constitute a safety margin, and it may have no more significance than that

S Udenfriend The extremes are the things one should consider, not the minor differences

D W Woolley I want to point out in view of what Dr Udenfriend said about behavior, that we attempted to reproduce the walking-backward LSD phenomenon in mice by giving 5-hydroxytryptophan It seemed like a good way to increase the serotonin in the brain The mice will not walk backward or behave as they do

CHAIRMAN I. H. PAGE. That is right

G. PINCUS. Won't LSD do the same thing as serotonin?

A. S. MARRAZZI. Yes.

B. B BRODIE: Does not anoxia usually result in convulsion?

S. S. KETY: Generalized anoxia may, but this is a very special type of phenomenon

B. B BRODIE: You get sedation with serotonin

H HOAGLAND The time course of these electric effects is interesting If you think of a dose producing a change in circulation locally, we ought to bear in mind that the inhibition of the spontaneous activity in the rat may last upwards of 15 minutes to an hour.

R W. GERARD: How soon does it start?

H. HOAGLAND: In a matter of a minute or less

G. H. GLASER Has the sensitivity to small amounts of barbiturates been tested?

S. UDENFRIEND We have no evidence for this

CHAIRMAN I. H. PAGE. We have no particular evidence either, although we have not especially looked for it.

R. A CLEGHORN. It might be interesting to try the Shagass sedation threshold test It is a fairly accurate measurement

CHAIRMAN I H PAGE. I do not know what that test is.

R A CLEGHORN A test which has been developed by Shagass in the Allan Memorial Institute for psychiatric patients He has been able to demonstrate that the different categories of mental patients have different sensitivity to barbiturates (sodium amytal) measured by the development of slurred speech and electroencephalographic changes It is an easy test to do in a properly equipped place and would give concise evidence

H. HOAGLAND I wonder if the implications from Dr Udenfriend's work on pyridoxine-deficient chicks would imply that perhaps the psychosis of pellagra might in some way be linked up with serotonin deficiency on the basis of the role *of the B vitamins in the relief of this psychosis I don't remember that pyridoxine* is specifically involved here, but certainly the vitamin B complex and one or two of its components and perhaps pyridoxine, have been of use in the treatment of this psychosis and here may be an opportunity to link a specific clinical psychosis with serotonin deficiency Is this something that makes any sense or not?

D W. WOOLLEY It is very sensible Dr Udenfriend has shown that the conversion of tryptophan to hydroxytryptophan and consequently to serotonin is dependent on a nicotinamide-containing enzyme system, i e, a TPN-system Lack of nicotinamide leads to reduction of TPN and would be expected to reduce *hydroxytryptophan production*

S. UDENFRIEND Epinephrine, norepinephrine, and histamine also require pyridoxine for their metabolism

H. HOAGLAND In pellagra one might then expect a serotonin deficiency Moreover, pellagra can be prevented in persons fed on corn, by including in the diet foods rich in tryptophan (Rosen, F, Huff, J, and Perlzweig, W *J Biol Chem* **163**, 343, 1946, Sarett, H., and Goldsmith, G, *J Biol Chem* **167**, 293, 1947) What about vitamin B6, i e, pyridoxine?

G H GLASER Specifically I don't believe pyridoxine is involved in the psy-

# On a Role for Serotonin and Norepinephrine as Chemical Mediators in the Central Autonomic Nervous System

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It is too often forgotten that the autonomic nervous system is not to be regarded only as an efferent system that carries impulses from the central nervous system to various organs Information originates from visceral structures, is transmitted by afferent nerves, and is integrated in the central autonomic nervous system. Impulses from the autonomic centers thus complete a multitude of reflex arcs. Some of the integrating centers are present at the level of the spinal cord, but the major ramifications of the central autonomic nervous system are in the brain stem, with the hypothalamus being the principal locus of integration of the entire autonomic system The central autonomic system, like the peripheral one, may therefore be considered not as a single division but as two distinct, mutually antagonistic systems, the parasympathetic and the sympathetic. Thus, it is known that the nuclei in the posterior and lateral part of the hypothalamus are sympathetic in their main connections, whereas the nuclei in the anterior hypothalamus integrate mainly parasympathetic functions. Even the cortex appears to provide integration of the sympathetic and parasympathetic functions and it is presumably here that the autonomic and somatic systems interlock.

Much is known about the chemical transmitters of the peripheral autonomic nervous system, but little is definite concerning the identity of mediators in the central autonomic system, although a number of workers have considered the possibility that serotonin (6, 9, 22, 25) and norepinephrine (24) might be central neurohormones We are going to propose the concept that serotonin is the chemical transmitter of the central parasympathetic system and norepinephrine the transmitter of the central sympathetic system. We plead guilty in advance to proposing a thesis that is admittedly oversimplified, but as a working hypothesis it has the merit of tying together a number of apparently unrelated observations and of suggesting experiments which might help in the under-

when given LSD following administration of 5-hydroxytryptophan. It would have been nice if they had done so.

S. UDENFRIEND: As to walking backwards, our mice don't walk backwards either.

F. ELMADJIAN: You presented data on the whole brain of the chicken. It seems to have a rather high serotonin content. Did you make a point of that?

S. UDENFRIEND: Yes. I was struck by that too, until I mentioned it to Dr. Anderson. She pointed out that the chicken has very little in the way of higher brain centers.

F. ELMADJIAN: Another question I would like to ask is that with the various tissues, you have shown a drop in the serotonin content with pyridoxine deficiency. Would you like to say something about the nature of what was left, whether it was bound material or free?

S. UDENFRIEND: I think any serotonin we find in tissue is bound.

H. J. KOCH, JR.: Has the serotonin level been studied in patients with regional ileitis and ulcerative colitis?

S. UDENFRIEND: Actually, Dr. Wiener in New York studied large numbers of different types of patients, and except for those with malignant carcinoid, no unusual levels were found.

6. *The addition of the substance to nervous tissue should have a demonstrable central effect.* When a large dose of serotonin is given intraperitoneally to mice, a small quantity of the amine penetrates the brain. The mice are sedated and are hypersensitive to the action of hypnotics (20)

7. *Inhibition of the inactivating enzyme should show a central effect* Acetylcholine, at a peripheral ganglion cell, is liberated by a nerve impulse and is generally considered to depolarize the synaptic membrane and then to be rapidly metabolized by cholinesterase. If a persistent concentration of free acetylcholine is present, a volley of postganglionic impulses may result However, if the ganglion cell is flooded by acetylcholine in high concentration, the persistent depolarization that results prevents activation of the postganglionic fiber by preganglionic stimulation (14). The same final effect is thus obtained by an excess of acetylcholine as by a substance which blocks the action of acetylcholine.

Similarly, in the central nervous system, serotonin may be considered to be released at synaptic junctions in minute amounts by a nerve impulse, and then be rapidly metabolized by monoamine oxidase As described later, reserpine may be considered to exert a persistent parasympathomimetic action by impairing the sites which hold serotonin in a bound form, resulting in a low but persistent concentration of serotonin in direct contact with central synapses.

Carrying over the analogy with acetylcholine, the question arises whether an excess of free serotonin would give the opposite results of a small quantity. Rabbits pretreated with iproniazid, a compound that inhibits monoamine oxidase and consequently the metabolism of free serotonin, and then given reserpine to release brain serotonin, show little if any decline in brain serotonin Instead of displaying the usual effects of reserpine the animals exhibit in a dramatic fashion the responses typical of LSD, including violent excitation, mydriasis, exophthalmus, piloerection, and other signs of sympathetic activity It seemed possible that the sympathomimetic effects were caused by combination of iproniazid and reserpine rather than by the serotonin that had been released and protected from the action of monoamine oxidase. This possibility was investigated by reversal of the order of drug administration, first giving reserpine and then iproniazid. Now the serotonin in brain is low and the animals exhibit the sedation and parasympathomimetic effects typical of reserpine (Table I).

An excess of free serotonin in the brain has also been produced by the administration of the precursor agent, 5-hydroxytryptophan. As de-

standing of the mechanism of action of centrally acting drugs such as reserpine, chlorpromazine, lysergic acid diethylamide (LSD), mescaline, and other phenylethylamine analogs.

It may seem surprising that serotonin rather than acetylcholine should be considered as the neurohumoral agent for the central parasympathetic system The reason why serotonin has been proposed will be obvious as the relationship between brain serotonin and the parasympathomimetic activity of reserpine is considered. It must be emphasized, however, that we are not suggesting that acetylcholine does not have an important role in central transmission, but only that its main action may not be in the central autonomic system. Indeed, as far as we know, there is little or no evidence indicating that acetylcholine is a transmitting agent in the central autonomic system.

It is an exceedingly difficult problem to prove that a chemical substance is a synaptic transmitting agent in the central nervous system. Recognizing this, let us consider some minimal criteria that a candidate compound must meet and how well serotonin satisfies these requirements.

1. *The substance must be present in the central nervous system* The presence of serotonin in brain was demonstrated by Twarog and Page (23) and Amin *et al* (1), using bioassay procedures. Later, using a specific fluorometric assay, its presence in brain was identified in our laboratory by more exacting physicochemical criteria including countercurrent distribution and activation and fluorescence spectra (2).

2. *The substance should be concentrated in the locale of its proposed function in the central nervous system* The substance has its highest concentration in the brain stem, especially the hypothalamus, where the major portion of autonomic integration occurs It is lowest in the cortex and cerebellum (1).

3. *An enzyme for the inactivation of the substance must be present.* Monoamine oxidase is present in considerable activity in all parts of the brain, but, as shown by Udenfriend *et al* (p 153 in this volume), is especially concentrated in the hypothalamus

4. *An enzyme for the synthesis of the substance must be present.* In general, as discussed by Udenfriend in today's conference and by Gaddum and Giarman (7), the concentration of serotonin and the activity of the synthesizing enzyme, vary in a parallel fashion in the brain

5. *An antagonist of the substance should exert a central action if it can penetrate the brain* LSD antagonizes the central effects of administered serotonin and evokes marked central sympathetic activity (20, 17).

reserpine effects its release. These terms in relation to serotonin are used to signify that the compound in tissues is not freely available to monoamine oxidase which otherwise would quickly destroy it. To explain this protection it could be assumed that the compound is anchored onto a

TABLE II

UPTAKE OF SEROTONIN BY PLATELETS OF NORMAL GUINEA PIGS AND OF ANIMALS THAT RECEIVED RESERPINE (5 MG. PER KG.) 16 HOURS PREVIOUSLY[a]

| Platelets from normal animals ($\mu$g per mg protein) | Platelets from reserpine-treated animals ($\mu$g per mg protein) |
|---|---|
| 1.45 | 0.19 |
| 1.34 | 0.21 |
| 1.48 | 0.11 |
| 0.86 | 0.20 |
| 0.60 | 0.13 |
| 1.35 | 0.10 |
| 1.39 | 0.14 |
| Average 1.21 ± .33 (SD) | Average 0.15 ± .05 (SD) |

[a] 4 ml. of platelet-rich plasma and 1 ml. of isotonic saline containing 25 $\mu$g of serotonin were incubated in siliconed beakers for 1 hour at 37°C. Values represent net increase in serotonin concentration.

TABLE III

INCREASE IN BRAIN SEROTONIN FOLLOWING ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN TO NORMAL RABBITS AND TO RABBITS THAT RECEIVED RESERPINE (5 MG. PER KG.) 16 HOURS PREVIOUSLY[a]

| Normal animals. $\mu$g serotonin per gram brain tissue | Reserpine-treated animals. $\mu$g serotonin per gram brain tissue |
|---|---|
| 1.55 | 0.19 |
| 0.75 | 0.21 |
| 1.00 | 0.58 |
| 1.53 | 0.24 |
| Average 1.21 ± 0.40 (SD) | Average 0.30 ± 0.18 (SD) |

[a] Rabbits were given 50 mg. per kg. of 5-hydroxytryptophan and were killed 1 hour later. Values represent net increase in serotonin concentration.

specific constituent of brain tissue. Reserpine could act by "denaturing" the storage site so that it can no longer localize serotonin. Another possibility is that serotonin is maintained within cells in a high concentration by means of an active transport mechanism with which reserpine can interact.

There is not yet enough available information to make it possible to choose between the two possibilities. The chief argument for a physico-

scribed in the report by Udenfriend, this compound rapidly penetrates brain, where it is decarboxylated to form serotonin. If the dose of 5-hydroxytryptophan is low, effects suggestive of sedation may sometimes be observed in the dog and the mouse, but with high doses of the amino acid, serotonin is formed in considerable amounts, and, presumably by saturating the storage sites, results in a high level of free serotonin. Again the animals are excited and behave as though they have received LSD

TABLE I

EFFECT OF IPRONIAZID ON THE CONCENTRATION OF SEROTONIN IN BRAINS OF NORMAL AND RESERPINE-TREATED RABBITS[a]

| Drug | Brain serotonin (μg per gram, average) | Effect |
|---|---|---|
| None | 0 55 | — |
| Reserpine | 0.10 | Sedation |
| Iproniazid | 0 63 | None |
| Iproniazid followed by reserpine | 0 42 | Excitement |
| Reserpine followed by iproniazid | 0 10 | Sedation |

[a] Rabbits were given 5 mg per kg reserpine intravenously Iproniazid (100 mg per kg ) was given intravenously 2 hours before or after reserpine Animals were sacrificed 1 hour after last drug administration

Our notions concerning the possible function of serotonin in the central nervous system arose from experiments which suggested that the pharmacologic actions of reserpine were related to its singular property of impairing the capacity of body cells to store serotonin It was shown that serotonin added *in vitro* to platelet-enriched plasma of normal guinea pigs is avidly taken up by the platelets In contrast, platelets from animals given reserpine 16 hours previously lose most of their ability to localize serotonin (Table II) Reserpine also affects the storage capacity of brain cells This is difficult to demonstrate with free serotonin since the amine does not readily cross the blood-brain barrier, but can be shown with 5-hydroxytryptophan which readily enters the brain where it is decarboxylated to yield serotonin The brain tissue of normal rabbits readily localizes considerable amounts of the serotonin thus formed, on the other hand, the brain of reserpine-treated animals can take up only a small amount of the amine (Table III).

We shall digress somewhat from the main topic to discuss the interesting problem of how serotonin is "bound" or "stored" by body cells and how

will be rapidly metabolized by the action of monoamine oxidase. In Fig. 1 is shown the depletion of serotonin in rabbit brain that results from the administration of 5 mg per kilogram of reserpine Brain serotonin is especially sensitive to reserpine and a decrease of about 75% occurs within 30 minutes. The maximal decline, approximately 90%, occurs in about one hour, and the resulting concentration remains low for 48 hours or more, gradually rising to attain the normal level in 5 to 7 days.

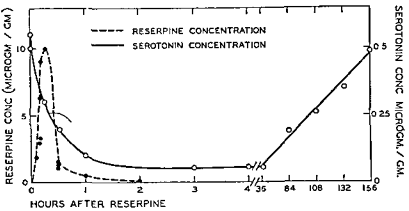


Fig 1 Concentration of reserpine and serotonin in rabbit brain at various times after intravenous administration of reserpine (5 mg per kg.) Each point represents a single rabbit

Following the administration of even large doses of reserpine, serotonin continues to be made in considerable amounts by the body. But with the impairment of its storage sites, free serotonin will leave brain cells in a continuous stream. The low levels of serotonin found in brain would then represent a balance between its rates of synthesis and destruction. Since the storage of serotonin is influenced to the same degree in different parts of the brain (Fig 2) the resulting level of free serotonin in brain should be highest in the brain stem where autonomic centers are concentrated and where reserpine is believed to exert its main pharmacologic action The low serotonin levels persist until the storage sites regain their capacity to take up serotonin, or until new storage sites are formed

It would indeed be surprising if the effects of reserpine were not in some way connected with the change in brain serotonin, considering the extraordinarily powerful physiological action that the amine exerts on many organ systems in the body (5, 13) Still, some consideration

chemical binding onto a cell constituent is the high concentration gradient of serotonin between rabbit platelets and plasma. Considering that there is about 3.5 $\mu g$. of serotonin per milliliter of rabbit blood and that virtually all of the amine is confined to platelets, the ratio of platelet concentration to plasma concentration may be in the order of several thousand. There is no known transport mechanism that can maintain such a high concentration gradient. On the other hand, other findings made it somewhat difficult to accept the explanation that serotonin is localized in platelets by physicochemical means. Attempts to demonstrate the actual presence of a complex in brain have been unsuccessful. For example, serotonin, unlike acetylcholine, is metabolized very rapidly in homogenates of various tissues. It is difficult to conceive that a serotonin complex with a very high association constant would split merely by homogenization of tissue cells Furthermore, after differential centrifugation of brain homogenate, serotonin was not found to be associated to a high degree with any of the particulate fractions.

Further data on the possible nature of the "binding" of serotonin by platelets have been obtained from studies *in vitro* showing that reserpine releases serotonin from a suspension of rabbit blood platelets in plasma (18). A concentration of the alkaloid as low as 0 05 $\mu g$. per milliliter of plasma liberates measurable amounts of serotonin, while 0 3 $\mu g$. per milliliter causes a maximal rate of release, about 50% in 4 hours The release process is not a simple displacement since one molecule of reserpine can liberate hundreds of serotonin molecules. The rate cannot be increased even if the concentration of reserpine is increased by one hundred times. This suggests that reserpine might rapidly inactivate the "storage" mechanism, and that the subsequent slow passage of the indole from platelets is a consequence of the slow penetrability of a poorly lipoid-soluble substance across a cell membrane possessing the characteristics of a lipoid barrier In contrast with this slow diffusion from the platelets of animals pretreated with reserpine, normal platelets (in the absence of reserpine) take up serotonin with considerable rapidity. This is further evidence in favor of a transport mechanism

It is obvious, however, that more work must be done on the fascinating problem of how this and other neurohumoral agents are held in a "bound" form

Let us now consider the consequences of the action of reserpine on the serotonin storage capacity of body cells A certain amount of the indole, depending on the dose of reserpine, will be liberated from intestines (15), platelets (21), and brain (16), as well as from gastric mucosa, and

sumed that this compound acts by blocking the action of liberated serotonin Of special importance is the unusual specificity of action among the *Rauwolfia* alkaloids (Table IV) in that only the tranquilizing *Rauwolfia* alkaloids cause an alteration in the level of brain serotonin (3). Methyl reserpate and reserpic acid, inactive hydrolytic products of reserpine, do not release serotonin. Other *Rauwolfia* alkaloids that are not sedative, even when structurally related to reserpine, do not liberate serotonin *in vivo* or *in vitro* from platelets. Of particular interest is the

TABLE IV

SEROTONIN CONCENTRATION IN RABBIT BRAIN 4 HOURS AFTER INTRAVENOUS ADMINISTRATION OF VARIOUS *Rauwolfia* ALKALOIDS

| Alkaloid | Dose mg/kg | Sedative action | Serotonin concentration μg/g |
|---|---|---|---|
| None | – | – | 0 57 |
| Reserpine | 2 | Active | 0 06 |
| Deserpidine (recanescine) | 2 | Active | 0 09 |
| Resecinnamine | 2 | Active | 0 10 |
| Methyl reserpate syringate | 3 | Active | 0 06 |
| Raunescine | 5 | Active | 0 07 |
| Methyl reserpate acetoacetate | 3 | Slightly active | 0 34 |
| Isoreserpine | 2 | Inactive | 0 48 |
| Isoraunescine | 5 | Inactive | 0 47 |
| Methyl reserpate | 2 | Inactive | 0 44 |
| Reserpic acid | 2 | Inactive | 0 52 |
| Reserpinine | 2 | Inactive | 0 49 |
| Serpentine | 2 | Inactive | 0.45 |
| Ajmaline | 2 | Inactive | 0 44 |
| Ajmalicine | 2 | Inactive | 0 55 |

inability of isoreserpine, a pharmacologically inactive stereoisomer of reserpine, and isoraunescine, an inactive isomer of raunescine, to release serotonin Methyl reserpate acetoacetate, which exerts only a slight though definite sedative effect, releases relatively small amounts of serotonin Rescinnamine offers a particularly good example of the relationship between the intensity of the pharmacologic effects and the liberation of serotonin. This compound induces relatively little sedation *in the mouse and correspondingly releases* only small amounts of serotonin in the brain of this animal. In the rabbit, in which it exerts marked sedative activity, much more serotonin is released

It might still be argued that the change in brain serotonin is not directly involved in the effects of reserpine but that the drug acts through

should be given as to whether the pharmacologic manifestations of reserpine are a consequence of the change in brain serotonin or merely concurrent with it.

There is now considerable evidence for a cause and effect relationship between the change in brain serotonin and the pharmacologic effects of reserpine. For example, we have shown that the sedative and potentiating effects of both administered serotonin and reserpine in mice are blocked by LSD (19). Again, as seen in Fig. 1, reserpine rapidly enters the brain and arrives at its maximal concentration in 10 to 15 minutes

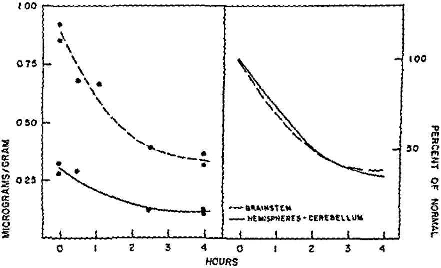


FIG. 2. Serotonin content of brain stem and of hemispheres plus cerebellum after intravenous injection of 0.25 mg per kg reserpine.

when the pharmacologic effects of the drug are barely apparent. The level in brain then declines rapidly and in 2 to 4 hours the drug is virtually undetectable when the pharmacologic effects have become maximal. The low levels of serotonin and the various pharmacologic effects including sedation, miosis, hypothermia, and hypotension persist for about two days. Also, of a number of centrally acting compounds, including barbiturates, analgesic narcotics, scopolamine, mescaline, Frenquel, chlorpromazine, meprobamate, and LSD, only the active *Rauwolfia* alkaloids were found to liberate serotonin. We were unable to demonstrate in the rabbit that amphetamine, even in convulsive doses, could affect brain serotonin as reported by Paasonen and Vogt in the dog (12). LSD does not prevent the release of serotonin, so that it may be pre-

Two hours after this latter dose, the sedative effect has disappeared, but most of the increment in brain serotonin is still present. It seems probable that the sedative effects are caused by free serotonin penetrating the brain and that it no longer exerts an action after it is bound to the nervous tissue. (2) Bogdanski in our laboratory has shown that when rats are injected with 100 mg per kilogram of the serotonin precursor, 5-hydroxytryptophan, the uterus takes up considerable amounts of serotonin, about 2.5 $\mu$g. per gram. This tissue in spite of its high serotonin concentration shows no contractile activity when suspended in Locke's solution. If a small amount of serotonin, 0.01 $\mu$g. per milliliter, is added, a strong contraction results. It is evident that the bound serotonin in the uterus is inactive, but a trace of free amine is highly active. (3) After reserpine administration there is a marked and persistent decline in the intestinal level of serotonin as the amine is released and metabolized. The increase in the motility of the intestines is maximal when the total serotonin (bound and free) is lowest. Since injected serotonin also increases the motility of the intestines, the action of reserpine is presumably due to free serotonin that is being continuously made by this organ and released because of the impaired capacity to store it. (4) Finally, if the decline in total serotonin were the determining factor in the action of reserpine, changes in the level of total serotonin in brain should parallel the pharmacologic effects. This is not the case. Thus rabbits are heavily sedated 30 minutes after reserpine administration (1 to 5 mg per kilogram) when the serotonin level in brain has declined to about 0.2 $\mu$g per gram of tissue (Fig. 1). Yet no sedation is apparent in 72 hours, when the serotonin level has returned to about 0.2 $\mu$g. per gram. This can be explained by assuming that free serotonin is the cause of sedation. Shortly after the administration of reserpine, serotonin is still being released and therefore some free serotonin is present, in 72 hours, although the total serotonin level is again 0.2 $\mu$g per gram, a negligible amount of free serotonin is probably present because the serotonin binding sites had recovered sufficiently to take up the indole as it is formed in brain.

Thus, it would appear that reserpine does not act *per se*, but by causing a continuous flow of highly active free serotonin which stimulates the synapses of the central parasympathetic division. As a result sedation, miosis, hypotension, hypothermia, ptosis, and other signs of parasympathomimetic activity are induced.

If we regard the central autonomic system as consisting of two antagonistic systems, parasympathetic and sympathetic, then it should be

a persistent metabolic product. This possibility is unlikely since administration of 1 or 5 mg. per kilogram of reserpine to rabbits produce the same intensity and duration of pharmacologic effects, and the time for the restoration of serotonin levels is the same with the two doses (Fig 3). If reserpine acted through a metabolite, the larger dose should have resulted in the formation of more of the metabolic product than the small dose and therefore have caused a more prolonged response.

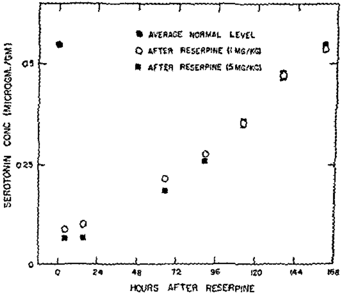


FIG 3 Serotonin content of rabbit brain at various times after intravenous administration of reserpine (1 or 5 mg per kg) Each point represents a single rabbit

The question now arises—are the pharmacologic manifestations of reserpine due to the decrease in total serotonin in brain, shown in Fig 1, or to the constant flow of free serotonin from tissue cells which make serotonin, but which have an impaired ability to store it? The second possibility is the more plausible, as in all experiments in which serotonin is used as a pharmacologic agent, only the free form seems to be active. (1) Reserpine-like effects which last for about 60 minutes are displayed in mice following the intraperitoneal injection of 20 to 100 mg per kilogram of serotonin Analysis of the brains removed from animals 15 minutes after the injection of 100 mg. per kilogram, show that small but significant amounts of the indole can penetrate the blood-brain barrier.

effects of LSD, only chlorpromazine antagonizes the LSD-like effects of excess serotonin. It would not be expected that reserpine, if it acts through free serotonin, would be a sedative agent in this situation since there is already too much free serotonin present.

The difference in the modes of action of the two tranquilizing drugs is best explained by assuming that they act on physiologically antagonistic systems in the brain, that is the central components of the sympathetic and parasympathetic nervous systems. If serotonin is the transmitter substance for the parasympathetic division, what is the corresponding one for the sympathetic? The distribution of norepinephrine in the brain as reported by Vogt (24) makes it the probable candidate. It has the same pattern of distribution in the brain as does serotonin. For example, its highest concentration is in the brain stem, especially the hypothalamus, it has a low concentration in the cerebral cortex and is virtually absent from the cerebellum

We are now in a position to think of the action of a number of centrally acting drugs, not in terms of their interference with enzymatic processes, but by acting somewhere within the neurohumoral frame of reference Reserpine, for example, may exert its parasympathomimetic effects by causing a constant flow of free serotonin to stimulate parasympathetic centers. LSD, a "serotonergic" blocking agent, may block nerve transmission in the central parasympathetic system by antagonizing serotonin at synaptic junctions The central sympathetic system would then be unmasked, resulting in a picture of sympathetic predominance.

Chlorpromazine could be considered to block norepinephrine at the synapses of the central sympathetic system, resulting in a net parasympathetic predominance Evidence points to chlorpromazine having little ability to block the peripheral effects of intravenously administered norepinephrine. This indicates that the lowering of sympathetic tone is central in origin. Indeed, it has been demonstrated that chlorpromazine, given intracisternally to animals, causes a decrease in blood pressure and a blockade of the carotid pressor reflex in doses too small to affect the pressor response of systemic injection of epinephrine (4) It has also been shown that chlorpromazine is inactive in the spinal dog in a dose that is hypotensive in the intact animal (8). Of considerable interest are the observations by Moran and Butler (11) who have shown that chlorpromazine sulfoxide, a major metabolite of chlorpromazine, produces sedation, orthostatic hypotension, and partial blockade of the carotid pressor reflex at doses which do not block the rise in blood pressure induced by injected epinephrine.

possible to induce sedation and reduced sympathetic tone by stimulating the parasympathetic centers or by blocking the sympathetic ones. Now we are in a position to discuss how reserpine and chlorpromazine may produce similar central actions, despite the fact that chlorpromazine does not act through serotonin. A number of observations make it clear that chlorpromazine acts by a different mechanism from that of reserpine.

The action of chlorpromazine is reversible, that is, no effects are seen after the drug has disappeared from the body. In contrast reserpine is a hit-and-run drug and its effects become most prominent in about two hours, when most of the alkaloid has been metabolized, and last far beyond the time the drug is detectable in brain. The findings with chlorpromazine are in accord with the conclusion that the drug acts directly, rather than through the release of serotonin.

TABLE V

EFFECT OF LSD ON ETHANOL POTENTIATION BY RESERPINE AND CHLORPROMAZINE[a]

| Drugs | Duration of hypnosis[b] minutes (average) |
|---|---|
| Ethanol (controls) | 40 |
| Ethanol + reserpine | > 300 |
| Ethanol + LSD + reserpine | 54 |
| Ethanol + chlorpromazine | 150 |
| Ethanol + LSD + chlorpromazine | 147 |

[a] Adult male mice were given the various drugs intraperitoneally. Reserpine (5 mg per kg) was administered 1 hour before ethanol (4 g per kg). Chlorpromazine (5 mg per kg) was given simultaneously with ethanol. LSD (10 mg per kg) was given in two divided doses 1 hour before and simultaneously with ethanol. Mice given ethanol alone served as controls. LSD by itself had virtually no effect on duration of hypnosis produced by ethanol.

[b] Time from loss to return of righting reflex.

The question may be raised whether it is possible that chlorpromazine acts directly on the same synapses as reserpine acts *indirectly*. This is unlikely because of other differences between the drugs. Both drugs markedly potentiate by a central action the effects of hexobarbital and alcohol, but only the potentiation induced by reserpine is blocked by LSD (Table V).

But the most dramatic difference between chlorpromazine and reserpine is shown when they are administered to rabbits exhibiting LSD-like effects evoked by an excess of free serotonin in brain (whether produced by iproniazid followed by reserpine or by 5-hydroxytryptophan). Although both drugs reverse the excitation and other sympathomimetic

which have a central action is the ability to produce hallucinations in man. This is true for LSD, mescaline, amphetamine, and ephedrine, although large doses are needed with the latter two compounds. It would seem that LSD may differ in two important respects from the phenylethylamine analogs. (1) Its action may be that of blocking central parasympathetic transmission by antagonizing serotonin and thus unmasking the sympathetic system, rather than directly stimulating central sympathetic synapses (2) Its activity is an order of magnitude greater than that of the phenylethylamine analogs. This latter characteristic of the compound has led numerous workers to think of the properties of LSD as a "psychotomimetic" agent as something new, and peculiar to this compound. The production of hallucinations by a variety of sympathomimetic compounds may well be an inevitable consequence of overstimulation of the central sympathetic system

It is possible that one of the important consequences of the numerous studies with LSD, mescaline, and the tranquilizing drugs will be a better comprehension of the control of homeostatic mechanisms by the central autonomic nervous system, rather than a more profound understanding of mental disease.

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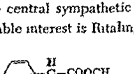
The actions of a number of other "adrenergic" blocking agents may possibly be interpreted in terms of inhibition of norepinephrine at sympathetic nerve centers. For instance, the dihydroergot alkaloids also produce sedation, and lower blood pressure and heart rate by a central action.

A number of effects of phenylethylamine analogs which can penetrate brain tissue can perhaps be explained in terms of their mimicking the action of norepinephrine at central sympathetic synapses. Even epinephrine and norepinephrine, if administered in large doses to animals can cause excitement and even convulsions, presumably due to small amounts of the substances penetrating the brain and activating the central sympathetic centers. The central effects induced by these catechol amines *are thus the opposite of those resulting from parenterally administered* serotonin. Amphetamine and ephedrine markedly stimulate the central nervous system and in addition stimulate the peripheral effects of norepinephrine. They have been considered to act directly on autonomic effector organs But there is difficulty in accepting this assumption in its entirety since the effects of ephedrine on certain effector organs are markedly lessened by sympathetic denervation in contrast to the enhanced effect of catechol amines. The usual explanation is that ephedrine and amphetamine act *indirectly* by *protecting* the adrenergic mediator from the action of monoamine oxidase at sympathetic nerve terminals. But it is now known that ephedrine and amphetamine exert only weak inhibitory effects on monoamine oxidase An alternative explanation is to assume that these substances, which readily penetrate the brain, mediate part of their peripheral effects through activation of central sympathetic centers Similarly mescaline (trimethoxyphenylethylamine), which causes central and peripheral sympathetic effects, may act by stimulating the central sympathetic centers.

A compound of considerable interest is Ritalin, which is also an analog of phenylethylamine



This compound is reported to block the effects of reserpine *in vivo* and has also been shown to evoke a number of peripheral sympathomimetic effects by acting within the central nervous system (10)

A characteristic common to a number of sympathomimetic compounds

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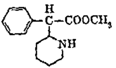

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## Discussion

S. S. Kety: The field of psychiatry, because of its tremendous complexity as opposed to the heart, a pump which everybody can understand just by looking at it, of course, is characterized by a great deal of theory and very little proof, and the thing that Dr. Brodie can contribute by means of his work, and that of his group, is to bring into this field more data than has been available before, better techniques, and newer approaches.

With regard to Dr. Brodie's theory on which he spent somewhat more time than he did on the data, I have a few questions that I want to ask him. These are rather minor questions, but they bothered me even though I recognize the value of a theory is just that it is speculative and leads to more research, it also provokes criticism and the need for answering criticism.

I was a little puzzled by the low brain serotonin in association with many other as yet unknown effects that reserpine may have, some of which may also be responsible for its behavioral and clinical effects. Dr. Brodie's group nicely demonstrated the fall in serotonin associated with reserpine administration, nevertheless, in the list of effects of reserpine, I found a number of similarities to those which Dr. Udenfriend described as being associated with the administration of 5-hydroxytryptophan and marsilid which presumably produce a high brain serotonin.

B. B. Brodie: According to our view, reserpine results in the presence of a persistent flow of serotonin from cells which have lost part of their capacity for storing the indole. Although the level of serotonin is low—and this is to be expected because free serotonin is rapidly metabolized by monoamine oxidase—the free amine stimulates the central parasympathetic centers, thus producing the signs typical of reserpine. Excess of free serotonin in brain tissue blocks its own action and as a consequence sympathetic centers are unmasked—in other words an LSD-like action is observed. It must be remembered that the administration of 5-hydroxytryptophan results in formation of serotonin in all body tissues. It is therefore difficult to interpret all the effects of 5-hydroxytryptophan, some of which may be due to the direct peripheral effects of serotonin.

S. S. Kety: If I may interrupt, those are *some* of the effects, but your other slide, which was on the effects of reserpine alone, listed also lacrimation, salivation, and muscular incoordination, which were reminiscent of the effects of high serotonin levels in the brain. I had one or two other questions. Where does LSD

now fit into the hypothesis and how is its role reconciled with Rothlin's data on brom-LSD, and also with Harris Isbell's recent as yet unpublished information that LSD effects are apparently potentiated by reserpine. Finally, Dr. Udenfriend indicated that you were going to give us some evidence that serotonin passes the blood-brain barrier in mice and I was wondering—

B. B. BRODIE: I will answer that last question first. There is no doubt that serotonin can penetrate into brain, though large doses of the indole must be given to show this analytically. After the administration of 100 mg per kilogram of serotonin to mice, intraperitoneally, the level of serotonin in brain increases by about 25%.

S. S. KETY. But there is then an increase in the brain?

B. B. BRODIE. Yes. It is of interest that 2 hours after the serotonin administration, the animals are no longer sedated, yet the brain serotonin level is still above normal. The serotonin increment is presumably now bound and therefore physiologically inactive.

D. W. WOOLLEY: May I say that those data do not correspond with our findings in mice? I just wanted to point out that apropos of increasing the serotonin in brain, we do not find it.

B. B. BRODIE: You have to give very large doses to show an increase by the spectrophotofluorometric procedure.

D. W. WOOLLEY. How large is what you call a large dose?

B. B. BRODIE: 50 to 100 mg per kilogram.

D. W. WOOLLEY: We gave 400 mg per kilogram.

B. B. BRODIE: There was a definite increase in brain serotonin in our mice that received the indole. How did you measure your brain serotonin?

D. W. WOOLLEY. By Dr. Udenfriend's method.

A. S. MARRAZZI. I was going to say this. Dr. Brodie has quite properly emphasized that the utility and the test of theory is its ability to fit data. We have a set of data that we would like him to fit. Using a much simpler indicator where the variables are somewhat easier to control, i.e., cerebral synaptic inhibition in the cortex of the cat, we find that there adrenaline and noradrenaline and serotonin instead of being different, are like mescaline and LSD and amphetamine, all of them

that the classical pharmacological notion of explaining the action of amphetamine and ephedrine has not yet been displaced, that is, they have an action on amine oxidase and actually produce their effects through the preservation of adrenaline.

One more point, the notion that reserpine and chlorpromazine compete with serotonin for receptors is somewhat borne out by the fact that they will not only prevent the action of serotonin-like substances, but when given in larger doses (in the case of reserpine it is only twice the dose, in the case of chlorpromazine it is 20 times the dose) they will of themselves have the same action as serotonin, that is, a synaptic inhibitory action.

This set of data does not meet this formulation at all. How does it fit your concept?

B. B. BRODIE. I am afraid that your experiments lead to one concept and ours to another, but I am sure that with additional studies the two sets of data will make sense. In regard to your remarks about the action of ephedrine and amphetamine, it is not possible to retain the classical picture of their acting through preservation of norepinephrine or epinephrine. These compounds are rather poor inhibitors of amine oxidase, compared to marsilid which incidentally does not act like ephedrine.

A. S. MARRAZZI: It does, it produces synaptic inhibition.

B. B. BRODIE: But it does not evoke sympathomimetic effects. Since you administer serotonin by intracarotid injection, and thereby bypass to a large extent localization of serotonin in platelets and breakdown by monoamine oxidase, is it possible that you are achieving in the transcallosum pathway an excess which would then act like LSD?

A. S. MARRAZZI. You are not going to drag that red herring in again?

B. B. BRODIE: I am afraid so.

A. S. MARRAZZI: As far as we can tell, the excess does not make any difference. We started with the lowest dose, 1 µg. The effect is always inhibition. Furthermore, although it is very easy for me to conceive of an excess of a stimulant having a different action, that is a paralytic action, therefore different from the action of a small dose, it is rather difficult for me to conceive of a primary inhibitor in larger quantities having an opposite action by the same mechanism, rather than by an action on a different group of cells. In other words, it is very difficult for me to conceive that small doses and large doses of serotonin are going to produce opposite actions by the same mechanism.

B. B. BRODIE: But our results indicate that serotonin is probably a stimulant of the centers of the central parasympathetic system. Instead of my being on the defensive—why don't your results fit ours?

A. S. MARRAZZI: You proposed the theory.

CHAIRMAN I. H. PAGE: Offense is the best defense.

B. B. BRODIE: A possible explanation of your results is that serotonin is not a normal neurohumoral agent in the transcallosal pathway and that you are measuring the pharmacologic effects of a "foreign" compound. I think you have to prove that serotonin is present in the transcallosal pathway.

A. S. MARRAZZI: It is present in the cortex. Inhibiting monoamine oxidase by intracarotid marsilid reproduces the action of serotonin, so presumably it is there.

S. S. KETY: I was wondering about the brom-LSD which was bothering me.

B. B. BRODIE: Brom-LSD is a potent antagonist of serotonin, as shown mainly with smooth muscle preparations *in vitro*. It does not have much effect *in vivo* as an antagonist of the central actions of administered serotonin or of reserpine. Furthermore, it produces little if any of the central sympathomimetic effects of LSD. Unlike LSD it does not counteract the ability of serotonin or reserpine to potentate the action of barbiturates. Perhaps it is metabolized too rapidly.

S. S. KETY: Rothlin indicated that brom-LSD does indeed get across the brain-

blood barrier because in the brain of animals that have received brom-LSD he is able to recover a serotonin-inhibiting material demonstrable in the bioassay So I think it does get across the blood-brain barrier, and still behaves like brom-LSD It is not all metabolized

B. B Brodie Then let us say that just because a compound blocks serotonin at one site, it does not necessarily do so at another Acetylcholine, for example, *is blocked by atropine at some sites much better than at others*

S. S Kety: You would say the smooth muscle effects of LSD are not necessarily the ones which explain its mental effects?

B. B. Brodie I would say that it might be best to have an open mind on the subject

Chairman I H Page Brom-LSD is not strictly without effect on the psyche If we give a large quantity of it by vein we get some very peculiar mental effects from it, so it is not quite a black and white issue as Rothlin indicated. He said there was no hallucinogenic effect

S. S. Kety What about sedation?

Chairman I H Page It has almost no sedation effects

S Udenfriend We have done some studies in carcinoid patients If you give 50 $\mu g$ of LSD to a carcinoid patient, the hallucinatory effects are the same as in the normal, but he gets marked peripheral effects from it. We have given 7 mg. of brom-LSD without being able to observe very much

A G Slocombe If one is to propose serotonin as a neurotransmitter, one must examine its properties as a depolarizing agent. The only evidence I know is the work done by Dr Twarog on the *byssus retractor*, a small muscle in the mollusc *Mytilus* In studying both contraction and demarcation potential, she found that acetylcholine produced contraction and depolarization, but when the acetylcholine was washed out the muscle was repolarized without relaxation When serotonin was then put on there was relaxation without any change in demarcation potential In this preparation, at least, serotonin is not acting as a depolarization or hyperpolarization agent Does anyone know of any preparation where serotonin does act on the resting potential?

O Diethelm I would like to make a brief remark to clarify the use of the word "hallucinations" In the present discussion we speak of visual hallucination, not auditory In schizophrenia the visual hallucinations are rare, but the auditory are frequent Visual hallucinations are found where there is damage to the sensory nervous system and in the presence of various drugs There has been much drug experimentation for nearly a century We have to be careful when we speak of hallucinations In offering descriptions of hallucinations it would help if one could know the type of visual hallucinations as far as color, size, movement, and other points are concerned Tactile hallucinations and olfactory hallucinations are also rare in schizophrenia and in the manic-depressive group They occur in the presence of various drugs and in metabolic disorders Here again, detailed observations would be helpful To illustrate, for instance, many of the tactile hallucinations occur in states of intense sexual excitement Reserpine may reduce sexual excitement, but there is a rather narrow margin in the sexually excited

person when reducing the excitement too suddenly Stimulation may then result in the same hallucinations. Chlorpromazine usually reduces the sexual excitement and does not seem to have the narrow margin

G. S. GORDAN: One of the places for discrepancy is the solvent in which reserpine is dissolved Dr. Brodie showed central nervous system stimulation or depression from the administration of reserpine under differing circumstances We, too, have found that the intravenous administration of reserpine in a dose of 5 mg. has a stimulatory effect upon cerebral metabolism of the schizophrenic patients. To our surprise, this "sedative" drug increased the rates of cerebral blood flow and oxygen and glucose uptake. We soon found, however, that the stimulatory effect could be produced by administering the polyethylene glycol solvent alone without reserpine.

# Adrenolutin as a Psychotomimetic Agent[1]

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The autonomic nervous system and adrenaline, one of its derivatives, are so closely related to the abnormal and normal physiology of psychiatric disorder and of anxiety that it requires no extraordinary feat of imagination to suppose they are causally related Chemicals which interfere with the utilization of adrenaline by receptors (21) or which disturb its enzymatic destruction (7, 14) modify psychological states When administered parenterally, especially by the intraventricular route, it profoundly changes the psychological condition of animals and humans (4, 5, 11, 12, 19) In addition, some substances which resemble adrenaline in structure have potent and interesting hallucinogenic and euphorient qualities (1, 13). Nevertheless, it is not permissible in my opinion to postulate that such a universal substance can account for unique phenomena Some of the derivatives of adrenaline may not be universal and perhaps may have the psychosis-producing quality postulated over the past century for the schizophrenic toxin.

The suggestion that adrenaline metabolites had something to do with schizophrenia was enunciated by Hoffer *et al* (9) who reported that adrenochrome, its first oxidized derivative, produced psychological changes in human volunteers which were a paler image of the mescaline phenomena This idea arose out of an earlier suggestion by Osmond and Smythies (15) that the schizophrenic M substance was structurally intermediate between mescaline and adrenaline The observation that all but one of the known hallucinogenic substances either were indole derivatives or were related to adrenaline, led to the conclusion that indoles related to or derived from adrenaline deserved psychiatric study.

Adrenochrome is a red substance which is unstable in the dry state and extremely labile in solution. There is no known method of assay for adrenochrome as it is completely destroyed by chemical manipulation (6). Fischer (6) found that allowing adrenochrome to stand in urine at body temperature quickly results in its destruction and it ap-

[1] Research supported by National Health Grants, Ottawa, Rockefeller Foundation, New York; and Saskatchewan Committee on Schizophrenia Research.

pears likely that appreciable destruction must occur in the urine of human subjects if it is present at any time. In some solvent systems adrenochrome forms an equilibrium mixture with adrenolutin, a reduced derivative of adrenochrome. Adrenolutin is a bright yellow crystalline compound more stable than adrenochrome in the solid state but not more stable in solution.

For the past year, we have been experimenting with adrenolutin as a psychotomimetic agent. Our first supply was obtained from Harley-Mason but subsequent lots have been synthesized by Pfizer and Company, New York.

## I PROPERTIES OF ADRENOLUTIN

The physiological properties are similar to those of adrenochrome. Eade (2) suggested that some of the activity of adrenochrome may result from its conversion into adrenolutin in the body. Generally it is more active than adrenochrome with respect to its hypothermic effect on rats (2), its ability to produce changes in cats when administered into the ventricles (18), and the toxicity as measured by the $LD_{50}$ (2). Recently, a surge of interest in adrenochrome has shown that it uncouples completely oxidative phosphorylation in hamster liver mitochrondria (17), modifies the DPN-TPN reduction system (3), flattens spontaneous EEG activity of cortical and subcortical centers in albino rats (20), in high concentration causes synaptic inhibition (8), and completely inhibits the decarboxylation of glutamic acid by brain tissue (10). It is possible that adrenolutin may share some of the properties with adrenochrome

## II. EXPERIMENTAL DESIGN

Adrenolutin and a placebo control (riboflavin) were administered to a series of volunteers in a double blind study. The volunteers were medical students, nurses, physicians, and some nonprofessional people None of the volunteers knew anything about the nature of the drug or what changes it would induce. They were, of course, aware that the study was part of a schizophrenia research program. The subjects were informed that they would be given two drugs, A and B, in order to determine which was the more effective euphorient During each experiment the subject was studied and interviewed by a psychiatrist and psychologist Observations were recorded and from the response of the subject, the research team guessed whether the subject had received drug or placebo Dosage was 50 mg by mouth. Experiments started at 6 P.M and were usually completed by 11 P M

## III. INTERIM RESULTS

Fourteen subjects received both adrenolutin and placebo and three received adrenolutin twice. Seven subjects received first adrenolutin followed the second week by placebo

*Subject one* when looking with his eyes closed at the stroboscope felt thrust into the visual field as if it were a void. He had a severe frontal headache and was dizzy, complaining, "It is like being dizzy but suffering from none of its effects." Next day he was dizzy all day, bored, and uninterested. Recently this subject has taken 500 mg of mescaline at 9 A.M. Beginning at 11 A.M. for the next four hours he reproduced the adrenolutin experience after which he had a normal mescaline reaction.

*Subject two* was a physician who showed no intellectual changes with adrenolutin He was however, argumentative, irritating, superior, and boastful and in an aggressive, overconfident manner described how he would change conditions With placebo, he was sociable, friendly, and quite different

*Subject three* became depressed which alternated with euphoria. His thought processes were sluggish and referential, and he made many errors in arithmetic. The subject giggled continually throughout the evening With placebo, there was slight euphoria but he was alert throughout the evening, able to solve all problems readily, and was very fatigued at the end of the experiment.

*Subject four* suffered from fatigue and boredom, complained of tension in the temples and difficulty in thinking The subject was irritable, hostile, and depressed Very little anxiety was evident. Under the stimulus of the stroboscope, the subject hallucinated pairs of matched eyes of different size slowly moving clockwise across the visual field She was fatigued and uninterested the next day With placebo, she was alert, highly interested in the experiment, suffered no visual changes, and no residual changes the following day

*Subject five* found conversation very difficult She felt insecure in argument and believed the argument was deliberately staged. She was easily confused, was critical of the experiment, and felt there was no purpose behind it Under the stimulus of the stroboscope, she hallucinated a forest of large trees of the type found in British Columbia with the sun glinting down on them She did not find this visual phenomena at all interesting The next day, her memory of the evening was very faint and she was very fatigued. With placebo, she was less distractable and found the proverbs much easier and was more alert. Anxiety was present throughout the evening.

*Subject six* felt within a half hour as if she had had two drinks of alcohol and was very tired as if she had not slept for fifty hours but was not sleepy. She complained of slight headache. She was argumentative, easily irritated, confused, very bored, but smiled excessively. No anxiety was present. Her response to the proverbs was very concrete There was no insight for her poor performance. The day following adrenolutin she was sleepy, fatigued, and nauseated. With placebo, she was very anxious and tense but her interest was high and there was some euphoria. She felt confused and was distracted readily but her performance was essentially normal.

*Subject seven* noted a marked decrease in anxiety later replaced by lethargy and apathy. Under the stimulus of the stroboscope, he had a sensation of two bright lights or two revolving centers of light as if looking into an inferno. Within an hour after taking adrenolutin, he developed a marked delusional belief that the two experimenters were communists. He did not communicate this feeling to the experimenters until a week after the experiment was finished. He felt very astute, clever, and superior to them He described himself as having supreme apathy. His intellectual performance was normal. With placebo, there was no change.

The next seven accounts of subjects refer to those who had placebo first.

*Subject eight.* There was no abnormality with placebo and normal anxiety was present. With adrenolutin, the response to proverbs was bizarre and response to calculations was very bad. The subject had difficulty focusing his eyes and noticed a slight visual change. He was very relaxed but also was very tired.

*Subject nine.* The only change noted with adrenolutin was a slight decrease in anxiety.

*Subject ten.* With placebo, she was anxious, felt that speech had changed, became apathetic and bored, and noticed alternate euphoria and depression. With adrenolutin, this subject was much less anxious She felt she was doing badly, was amused by this, and giggled a great deal. Under the stimulus of the stroboscope, she noticed a rose pattern There was no fatigue. She felt the experiment was silly. There was a marked decrease in intellectual performance. On returning home that evening, she was relaxed and talkative and, according to her roommate, markedly changed.

*Subject eleven.* With placebo, she was anxious, bored, and complained of difficulty in thinking and was easily confused The following day,

she was irritable. With adrenolutin, she felt very relaxed and flat She noted difficulty in focusing her eyes when looking at things. Next day the memory of the evening was faint, as if it had occurred three months before.

*Subject twelve.* On placebo, he was very anxious and felt as if he had had a shot of whisky. There was a loss of interest and great fatigue The next day, he had slight euphoria With adrenolutin, he noted frontal headache On reading, he reported that the words registered as words, not as phrases, and he had difficulty focusing his eyes. He felt mentally relaxed but physically tense and reported that he must have had a sedative drug This subject was invited to visit his fiancée that evening. On arriving at his fiancée's home, he spent the evening eating a sandwich, reading the newspaper, and listening to the radio. He completely ignored his fiancée. She found this very disturbing and became quite annoyed He realized she was disturbed but felt very aloof and impersonal especially since he knew she was in the wrong. This was the first time that this had ever happened to him. He was completely without sexual interest. The next day, he had planned to study but was unable to get going. He was irritable, tense, fidgety, and unable to concentrate all day.

*Subject thirteen* He was very anxious with placebo and noted some difficulty in thinking. He appeared indifferent, uninterested. With adrenolutin, he had some memory difficulty. Noticed no tension at all during the evening and was perfectly relaxed.

*Subject fourteen.* There was very little change except that he was slightly less tense with adrenolutin.

The next three subjects had adrenolutin twice.

*Subject fifteen* The first time, he complained of feelings of numbness in his limbs, headache, and a feeling of wooziness. He felt as if he had had a great deal of alcohol and his speech was very slurred. There was a marked decrease in insight and a very bad intellectual performance He was able to understand only four out of twelve proverbs. The second time he was brighter and more alert than the previous time and also more relaxed. His intellectual performance was better and he was able to solve eight out of twelve proverbs. He reported that both experiments were identical although to the observers there was a much greater reaction the first time

*Subject sixteen.* The first time all anxiety was gone by 6 30 A few minutes later, she felt lightheaded. There was ringing in her ears and a fuzzy feeling in her stomach She found herself unable to follow

*Subject six* felt within a half hour as if she had had two drinks of alcohol and was very tired as if she had not slept for fifty hours but was not sleepy. She complained of slight headache. She was argumentative, easily irritated, confused, very bored, but smiled excessively. No anxiety was present. Her response to the proverbs was very concrete. There was no insight for her poor performance. The day following adrenolutin she was sleepy, fatigued, and nauseated. With placebo, she was very anxious and tense but her interest was high and there was some euphoria. She felt confused and was distracted readily but her performance was essentially normal.

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overproduction of adrenaline and some permeation into the central nervous system.

The clearest reactions were obtained when adrenolutin was given the first time as here there was a combination of high initial anxiety plus adrenolutin. On the few experiments with double adrenolutin, the experience was usually more dramatic the first time, although the second experiment tended to reproduce what had happened the first time.

Between October 4th and December 19th, 1955, thirteen subjects were processed. Of thirty-two possible placebo predictions, twenty-one were correct and eleven incorrect. Of twenty adrenolutin predictions, fourteen were correct and six were incorrect. Chi square is over 5.0. Between January 9th and April 13th, 1956, twelve subjects were processed and with these we were not able to differentiate between drug and placebo. In the first thirteen subjects, only one subject out of ten who received the drug failed to react whereas in the second period, five out of ten failed to react.

The stipple test (which will be discussed further on) in the first eleven subjects predicted one wrong and ten correct, and in the second ten subjects, six wrong and four correct. This difference is significant. The stipple test predicted on the first sixteen subjects given placebo, four wrong and twelve correct, and the second sixteen subjects, six wrong and ten correct. It therefore appeared certain that either we had lost the ability to discriminate or that the drug had deteriorated to the point where it was not producing its effect. The decrease in clinical and in stipple test discrimination supports the second point of view. Slowly over the past few months adrenolutin has become much darker in color.

Fischer (6) recently found that adrenolutin incubated at pH 2 and body temperature is quickly destroyed It therefore appears likely that the oral administration of adrenolutin is not the best way and that a fair quantity is destroyed in the stomach This is a very valuable point and perhaps explains the marked difference in response of the men and women to the drug. Of six women given drug not one failed to react but out of eleven males given the drug, five failed to react. Perhaps this is due to the decreased acidity of the female gastric contents.

## V. THOUGHT DISORDER

The presence of thought disorder and decreased intellectual performance was judged by clinical impression of performance during discussion, argument, and questioning and by psychological tests The volunteer was questioned while under stress (due to the distraction

conversation and noted strong visual changes. She noted that the examiners' faces were changing and she felt that they were looking down upon her from a great distance. She showed referential thinking. Within an hour and a half, she had some slight nausea. Visual disturbances of words were present when looking at a printed page in that the words were hopping up and down. She felt very reticent about her feelings and found it difficult to communicate to us. She was bored and fatigued later on. She performed much below her normal intellectual level The second time, there was a reproduction of the first week's experience but the changes were not as intense. She had the same visual changes, felt suspicious of us, but did not have the fuzziness in her head which she had previously noted. On the next day after the first experiment, she was disinclined to work and felt fatigued and sedated until about noon when she suddenly recovered. After the second experiment, there was no sudden recovery and she suffered some effects of the drug the whole day.

*Subject seventeen.* The first time, very little change was noted. The only possible clue of drug activity was the fact that there was little anxiety in the subject coming in for the experiment. The second time, he was more relaxed than the first week but suffered a marked deterioration in intellectual performance The first week, he scored ten out of twelve, and the second week, seven out of twelve proverbs. On the comprehensive subtest of the Wechsler-Bellevue, the score dropped from eighteen to fourteen the second week. He was not made tense by questions the second week and was not as self critical of his answers

## IV. ABILITY TO DETECT ADRENOLUTIN AND PLACEBO

During the double blind procedure, it was very difficult to predict whether the subject had received placebo or adrenolutin This is not unexpected. Drugs such as mescaline and lysergic acid diethylamide impose a characteristic pattern upon the subject which is usually easily detectable. Adrenolutin does not impose any characteristic change upon the volunteers but by interfering with basic thought and perceptual processes allows each individual to react in an unpredictable way. Some of the subjects who received placebo the first time were most anxious; this was ascribed to adrenolutin. This substance is a possible metabolite of adrenaline. If therefore there is a substantial conversion of adrenaline to adrenolutin, the subject may react as if adrenolutin had been administered. Feldberg and Sherwood (5) suspected that experimental fatigue associated with marked anxiety might be associated with

them became flat and apathetic. The decrease in anxiety was accompanied by marked feelings of relaxation, by feelings of being at ease, and by lack of fatigue

The stipple test (16) was given to all subjects It was analyzed by calculating the variability in time required to scan the first twenty-five lines compared to the last twenty-five lines With adrenolutin, the variability was less during the latter half of the test. With placebo, it was greater. This simple paper test predicted correctly fourteen out of twenty-one adrenolutin experiments and twenty-two out of thirty-two placebo runs. It differentiated drug from placebo at one per cent level

Ten errors were made with placebo per test compared to thirteen errors for adrenolutin

## VII. LEVEL OF INTEREST

With placebo this was usually high and sustained. With adrenolutin, it was high at the beginning of the test but then decreased as the anxiety decreased. Many subjects noted marked apathy and general lack of interest which often lasted twenty-four hours.

## VIII VISUAL CHANGES

These were not invariably present but certain changes which were reported for adrenolutin did not occur under placebo. There were hallucinations under impact of the flashing light, difficulty in focusing on objects, feeling that two sources of light were present, a feeling that faces had become sinister and distorted, words were hopping on the page, inability to read words in groups as phrases

## IX. INSIGHT

In at least half the subjects who suffered a clear change, there was no accompanying insight Most ascribed more active changes to placebo when they described the usual accompaniments of anxiety. The most common reply to the question "has anything happened" was that nothing had or that there was a change due to the situation To many, placebo was the better euphorient None claimed adrenolutin as a euphorient, although some appeared more euphoric.

## X. DISCUSSION

The most striking changes occurred in the area of thought disturbance in the presence of reduced anxiety and decreased insight. Perceptual changes were not unusual but not as frequent as with mescaline or

caused by application of the EEG electrodes). The interviewer attempted to antagonize the subjects by critical argument. During placebo experiments, the subjects performed well although if extreme anxiety were present, a decrease in ability was noted. With adrenolutin, more often they were irritable and hostile, and failed to follow logical argument Some withdrew by becoming giggly or silly or by bringing in inappropriate statements. Little anxiety was created by discussion. Intellectually superior subjects showed little impairment of thought but did show changes in affect and in their relationship to the investigators.

Subjects were asked to define twelve well-known proverbs. Out of thirteen subjects, eight were able to solve fewer proverbs with adrenolutin, two were not impaired, and three were better. Qualitatively, they were more roundabout, more concrete, and more argumentative, often disagreeing with the proverbs Some subjects were more personally involved with the proverbs.

Adrenolutin caused a mean decrease on the comprehensive subtest of the Wechsler-Bellevue from fifteen to thirteen. On similarities, very concrete responses were found with some adrenolutin subjects but there was no decrease in the mean scores

Ability to describe subjective changes was decreased with adrenolutin and the description of the visual pattern induced by the stroboscope was less rich. Subjectively as many subjects with placebo complained of thought impairment. However although this was accountable by anxiety with placebo, they were not anxious with adrenolutin.

Orientation and consciousness were normal. Memory was normal with two exceptions. Paranoidal thinking was very marked in three instances and referential thinking slight in several other instances

Using the Watson-Glaser Critical Thinking Test (22), there was a slight decrease in total and in the subtotals with adrenolutin

In calculations, six out of twelve subjects with adrenolutin performed badly, with placebo, *only two With adrenolutin five out of twelve were* much worse, none were worse with placebo

## VI ANXIETY

All the volunteers showed anxiety especially during the first experiment After administration of placebo, anxiety decreased or increased but did not disappear. It was easily aroused by testing and questioning Strong anxiety was usually followed by strong fatigue toward the end of the experiment. With adrenolutin, most subjects noted a marked reduction in anxiety, which was confirmed by the investigators. Some of

DISCUSSION

S. UDENFRIEND I have two or three questions. First, you did not mention much about animal work. I wondered whether you cared to state whether anything really significant can be produced in animals The second concerns the effects on enzymes You did not mention the concentrations Also, it is not surprising that this compound would act on enzymes It is so active a molecule that it would interact with almost anything I think it is hardly specific.

For the final point I again have to bring up a disease, in this case pheochromocytoma I wonder if Dr Page would also mention his studies. Here you have a disorder in which there is huge overproduction of epinephrine and norepinephrine. Yet these patients, at least the three or four that we have seen, have pretty normal backgrounds Apparently the mere presence of large amounts of adrenaline does not serve to produce central disturbance Again I wondered what dose of adrenolutin was used in man

A HOFFER Fifty milligrams by mouth I will take the questions in order The animal work, recently published by B E Schwarz *et al.* in the *Arch Neurol Psychol* (*75, 83, 1956*) *used the Sherwood's technique of placing the drug in* the lateral or third ventricle I know Marrazzi has reservations about drug action placed in this area of the brain They tested cats using built-in electrodes making comparative studies with mescaline, adrenochrome, adrenolutin, and ergotamine

S UDENFRIEND· It did not act when given intravenously or orally?

A HOFFER I don't know When placed in the ventricle they found quite large changes They found that the most active compounds were mescaline, adrenochrome, and adrenolutin

A. S. MARRAZZI Dr Hoffer did mention the adrenochrome data on synaptic transmission, i e , a potency of about 1/2000 of the activity of serotonin as we test it

A HOFFER Regarding the enzymes, I think the quantity in one study I know of, using inhibition of decarboxylation of glutamic acid was 5 μg of adrenochrome which prevented decarboxylation of the glutamic acid

That is a good question about the pheochromocytoma. In my opinion schizophrenia is not caused by the overproduction of adrenaline, but by a malhandling of adrenaline formed Many people may suffer from too much adrenaline who will not be schizophrenic They must have something that will convert the adrenaline into another compound There is one case reported in the British press of a person, a paranoid schizophrenic, who after operation on the adrenals became normal

S S KETY The patient who had pheochromocytoma with schizophrenia would be a good patient to study and find out whether there was exacerbation of psychotic symptoms associated with the circulatory paroxysms

A HOFFER I don't know This was a study by Dr. Brewster I don't know whether he made many careful observations on the psychological state Most surgeons are not too concerned about the psychiatric changes present in their patients

CHAIRMAN I H PAGE That is the understatement of the day

LSD. Striking examples of marked personality change occurred in a few.

Adrenolutin is quite unstable. Oral administration has produced change with undeteriorated substance but becomes less effective as the compound decomposes. Intravenous administration has not been tested but adrenolutin ought to be twice as effective as adrenochrome.

The changes described are not those found in chronic schizophrenia. They do however fall into the area of the primary symptoms described by Bleuler. The most likely candidate for M substances from our point of view is either adrenochrome or adrenolutin (or an equilibrium mixture) until other adrenaline derivatives are isolated and synthesized.

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occur in, in the urine My point is that it takes anywhere from a year and one-half to two years for a schizophrenic to be ill before it is recognized by his family or physician, so there is a long enough period in which one could assume accumulation of some product

B B BRODIE: But adrenolutin is an unstable compound

A HOFFER: I have not said it is unstable in the body. We know it is unstable *in vitro*, in urine There is no reason to believe it is as unstable in the blood. In fact, there is reason to believe that in the blood it is quite stable It is stabilized by the proteins, glutathione, and ascorbic acid which are present there. We don't know. The more serious objection is that we don't know whether adrenolutin occurs naturally in the body

As to the question of pheochromocytoma, I don't know if there are changes The only people who have anything to do with pheochromocytoma are the internists who are not equipped to do very careful psychological tests on patients to see if their thoughts are impaired I would think if the patients are studied thoroughly by a good clinical psychiatrist and psychologist, you would be able to show changes on paper and pencil tests

G S GORDAN Many patients with pheochromatocytomas are mistaken for victims of anxiety states and are seen by psychiatrists for years.

A HOFFER. Not schizophrenic, however, but perhaps the early manifestations of schizophrenic illness. It is very difficult to define thought disorder It depends upon the psychiatric school to which you belong Some psychiatrists think it is a thought disorder Others think not I use it in the way Bleuler used it, to indicate disturbance, the dissociation towards concrete thinking. The person loses the ability to abstract If you use as a simple question, "how does a dog resemble the lion," he does not abstract the thought Both animals have eyes, or fur, etc That is a crude example of what I mean by a thought disorder, which is an inability to abstract information.

MEMBER Seen in brain damage?

A. HOFFER Yes, seen in many psychiatric disorders

F. ELMADJIAN In reply to Dr Brodie's remark about infusion of norepinephrine, I would like to make a few comments about methods and values and discuss briefly some experiments we have done with $C^{14}$-adrenaline at the Worcester Foundation.

With respect to methods, practically everybody working with the fluorometric method of Weil-Malherbe for adrenaline and noradrenaline, have had great difficulty I suggest that any data presented by this method requires cautious interpretation Based on our experience and the experience of others, it would be safe to pay more heed at present to bioassay methods if one wishes to consider the establishment of physiological formulation from the data obtained. The von Euler modification of the Lund method for urine determination is not bad, but there are still some marked discrepancies when compared to the bioassay values

To return to the adrenaline and noradrenaline studies which we have recently published Only 0 5 to 2% of the infused adrenaline appears in the urine as biologically active adrenaline Approximately 99% of the adrenaline infused is apparently destroyed Of the noradrenaline infused 3 to 5% appears in the urine

B. B. BRODIE: Am I right in assuming that this general idea started when you found that in certain patients epinephrine produced hallucinations?

A. HOFFER: That was one of the factors, but not the only one There is not a substantial body of evidence to show that adrenaline produces hallucination, but there is indication that certain types of adrenaline may produce certain changes

B. B. BRODIE: Large doses of a number of phenylethylamine analogs, including amphetamine, ephedrine, and mescaline, produce mental effects, including hallucinations. The mental effects may be simply the result of excess central sympathetic stimulation. The dose of adrenolutin you gave—50 mg I believe—makes one pause a little. Wouldn't it take a very long time to have that much metabolite form from epinephrine or norepinephrine in the body? You could argue that adrenolutin might localize somewhere in the body, but is this likely since it is such an unstable substance? All in all, it does not seem very plausible to me that schizophrenia results from a metabolite of epinephrine. Further information I would like to know concerns whether any of the subjects exhibited sympathomimetic effects when given adrenolutin? Were their pupils dilated?

A HOFFER. No autonomic changes whatever were seen.

B. B BRODIE. A final point. It is obvious that the cortex must be a complicated organ, and to try to explain memory, creativeness, and character in chemical terms is well beyond our present sights It may conceivably be a harmful oversimplification to try and explain a breakdown in cortical function in terms of an aberrant metabolite wandering about the brain. It is possible that subtle changes in proteins have occurred, changes that may not be explainable in the same frame of reference as the usual crude chemical alterations observed in biochemistry When I think of the complexity of the events that account for my remembering what you have just said, it is difficult for me to think that the breakdown in the brain is due to merely throwing a monkey wrench, like adrenolutin, in the machinery

G. H GLASER I would like to continue that point a little bit It is worth while still to differentiate between schizophrenia, the psychiatric disorder as it occurs naturally, and the schizophrenic-like psychoses that are produced by drugs I wonder how you differentiate between the thought disorder as you describe it and, for example, the mild thought disorder that one might see in relation to atabrine toxicity in which there is a superimposed schizophrenic-like symptomatology I think this is very fundamental to this whole consideration of drug-produced psychoses or hormone-induced psychoses

A HOFFER Going back to Dr Brodie's questions, I don't think that we actually know how much adrenaline can be produced by the body It is a serious objection to the theory to assume that adrenaline can produce 50 mg of adrenolutin in the body

B B BRODIE It would be of interest to know how much norepinephrine is formed in the body each day One could infuse the hormone, intravenously, over a long period of time and measure the fraction that is excreted unchanged By measuring the normal excretion of norepinephrine, before infusion, it should be possible to calculate the approximate amount of the hormone formed daily in the body.

A. HOFFER: The interesting point there would be to know what form metabolites

# THE THYROID AND BEHAVIOR

undestroyed. Quite clearly something has happened to these compounds. They do not appear in any sizable amount as biologically active material in the urine

S UDENFRIEND: Are they hydrolyzed?

F. ELMADJIAN: They were hydrolyzed We then infused $C^{14}$-adrenaline in the form of DL bitartrate in four separate experiments. It can be stated that 85 to 95% of the total count infused appears in the urine in 30 hours With respect to the biological activity present during the same period the experiments confirmed our previous infusion studies: only 0 5 to 2% of the biologic activity appeared

In relation to the discussions on the length of time for the excretion of adrenaline in pheochromocytoma, I would like to point out that adrenaline infused is excreted quite rapidly. In the first 4 hours after the infusion of $C^{14}$-adrenaline, 60% of the infused count appears in the urine

If these be the facts, as we think they are, then some of the ideas having to do with possible compounds being metabolized from secreted adrenaline and the possibility of their accumulation over a long period of time causing hallucinogenic effects, etc., are interesting formulations, but the data so far reported do not support the idea that much accumulation can take place

H. HOAGLAND In relation to the possible accumulation of adrenaline or its metabolites over a period of time, it seems to me that if there is a disturbance of appropriate enzyme balance, one might expect abnormal products of adrenaline or noradrenaline would be formed continually and metabolized to maintain steady-state levels different from those normally present If some intermediates build up abnormally, as a result of too rapid formation or too slow degradation, this would fit in with Hoffer's point of view in that the level of such metabolites could be abnormal as a result of disturbed steady-state chemical kinetics. The argument from infusion studies would not apply to this situation

# Metabolism of L-Thyroxine and L-3:5:3′-Triiodothyronine by Brain Tissue Preparations[1]

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The thyroid hormone has been known for many years to have a marked effect on the development and function of the central nervous system (14). Thyroxine and 3:5.3′-triiodothyronine increase the excitability of the brain, as measured by the threshold for electric shock seizures (22, 42). Both hormones also influence the electrolyte distribution and, in particular, the distribution of sodium in the brain tissue (42). The thyroid hormone also has marked effects on the electroencephalogram, and in the absence of thyroid hormone profound changes occur in cerebral vascular resistance and cerebral blood flow, changes in mental activity and mood, and even psychosis may be associated with either over- or underactivity of the thyroid (3, 10, 12, 13, 36) There is, however, no change in oxygen consumption in brain tissue removed from either thyroidectomized or hyperthyroid rats (6), although there is a slight change in succinoxidase activity (4).

In the central nervous system itself, there is a slight concentration of thyroxine and triiodothyronine when measured with radioisotopes (11, 16, 18, 19). This amount can be significant in view of the blood-brain barrier effective for most substances of physiological activity (17). Recently, thyroxine has been demonstrated in human cerebral spinal fluid and, in addition, an unidentified metabolite of the thyroid hormone has also been found (2). This metabolite was not found in blood and hence it seems possible that it was formed in the central nervous system itself.

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purity of each sample was ascertained by paper chromatographic analysis employing at least two different solvent systems. Care was taken to avoid the inclusion of any *n*-butanol in the substrate solutions as this solvent was found to interfere with tissue activity. Nonradioactive samples of L-thyroxine and L-3.5.3'-triiodothyronine were dissolved in the minimum quantity of 50% propylene glycol and then diluted with the isotonic suspension medium

## 4 Control Experiments

Each new series of incubations was accompanied by a set of at least two of the following control incubations where the substrate was incubated with: (a) suspension medium alone, (b) tissue system with *n*-butanol (final concentration 5%), (c) boiled tissue system (20–30 minutes boiling) The very small change in the final composition of radioactive components after incubation did not vary appreciably between the different controls and the final values were averaged.

## 5. Analytical

The brain slices were analyzed either separately from the incubation medium or after homogenizing them in the same medium All extraction procedures generally used for iodinated compounds were avoided. Paper chromatography (Whatman No 1) was used for both qualitative and quantitative determinations of iodinated compounds in each sample (26, 27)

At least two of the following solvent systems were used for the analysis of any one sample

1. *n*-butanol-acetic acid-water (78 10 12, v/v)
2. *n*-butanol-dioxane (80 20, v/v) saturated with 2*N* $NH_4OH$
3. collidine-water (100.35 5, v/v) in atmosphere of $NH_3$
4. ter-amyl alcohol saturated with 2*N* $NH_4OH$

Each sample for chromatographic analysis was supplemented with 10–25 $\mu$g of monoiodotyrosine, diiodotyrosine, 3.5 3'-triiodothyronine, and thyroxine as carriers. The final positions of these was revealed by ninhydrin or the Pauly reaction In some experiments, 25 $\mu$g of each of the propionic, acetic, and formic acid analogs of thyroxine and 3·5.3'-triiodothyronine were also added as carriers. Added iodide (5 $\mu$g.) was revealed with 0 5% $PdCl_2$ The radioactivity in each chromatographic strip after development was determined with an automatic recording counting rate meter. The percentage distribution of radioactivity in the

Considering the above facts and, in particular, the last observation, the present work was undertaken to define the activity of brain tissue in the metabolism of L-thyroxine and L-3:5:3′-triiodothyronine.[3]

## I. METHODS AND MATERIALS

### 1. Tissue Preparations

Unanesthetized adult male or female dogs (5–10 kg.), male or female rats (75–125 g.), and 2-week old male chicks were sacrificed by exsanguination or decapitation. Whole brains were collected in crushed ice or frozen in dry ice and, in the case of the rat and chick, brains from 15 to 30 animals were pooled. Brain slices were cut freehand from 0.5 to 1.5 mm. in thickness, and suspended in the bicarbonate-Ringer solution of Krebs and Henseleit (20) in the proportion of 1 g. wet tissue weight/5 ml. suspension medium. Tissue suspensions were prepared by homogenization in a Waring blendor run at a reduced voltage (30–40 volts), the concentration of the suspension being variable: 1 g. tissue/3, 5, 10, 15 or 30 ml. medium. The modified composition of the brain *suspension medium was (8):*

| | |
|---|---|
| 0.12 *M* NaCl | 0.0012 *M* $MgSO_4$ |
| 0.004 *M* KCl | 0.025 *M* $NaHCO_3$ |
| 0.0004 *M* $KH_2PO_4$ | 0.0005 *M* $CaCl_2$ |

### 2. Incubation

Incubations were carried out at 37.4°C. with continuous shaking of containers. Most incubations, unless specifically mentioned as otherwise, were carried out in an atmosphere of 5% $CO_2$-95% $O_2$. A 5% $CO_2$-95% $N_2$ mixture was used for anaerobic incubations. In the case of tissue suspensions or homogenates, aliquots were drawn from each flask at intervals varying from 15 minutes to 12 hours of incubation, while for slices one incubation flask was allotted to each time interval.

### 3. Substrates

L-Thyroxine and L-3:5:3′-triiodothyronine labeled with $I^{131}$ in the 3′:5′- and 3′-positions respectively (average specific activity: 40–60 $\mu$c/$\mu$g.) were obtained from Abbott Laboratories (15). Prior to use, the

[3] The following abbreviations are used in the text: $T_4$ = thyroxine, $T_3$ = 3:5:3′-triiodothyronine, TETRAPROP = 3:5:3′:5′-tetraiodothyropropionic acid, TETRAC = 3:5:3′:5′-tetraiodothyroacetic acid, TETRAFORM = 3:5:3′:5′-tetraiodothyroformic acid; TRIPROP = 3:5:3′-triiodothyropropionic acid, TRIAC = 3:5:3′-triiodothyroacetic acid, TRIFORM = 3:5:3′-triiodothyroformic acid.

TABLE I

DEGRADATION OF L-THYROXINE AND L-3 5 3'-TRIIODOTHYRONINE BY "CONCENTRATED" TISSUE PREPARATIONS (1 G TISSUE/3 ML. MEDIUM) OF DOG BRAIN

| Tissue preparation | Incubation[a] | L-Thyroxine, 8 × 10⁻⁹ M % Total $I^{131}$ distributed as[b] | | | | L-Triiodothyronine, 8 × 10⁻⁹ M % Total $I^{131}$ distributed as[b] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_4$ | $I^-$ | O[c] | Unidentified | $T_3$ | $I^-$ | O[c] | Unidentified |
| Controls | Aerobic | 85 6 | 12.1 | — | 2 3 | 87.6 | 9.2 | + | 1 0 |
| | Anaerobic | 83 5 | 13.4 | 1 0 | 2 1 | 91.1 | 8.7 | + | — |
| Slices | Aerobic | 26 9 | 69.3 | — | 2 2 | 28 3 | 65 8 | 1 5 | 1 6 |
| | Anaerobic | 25 7 | 68 6 | + | 4.4 | 32 5 | 63 6 | 2.1 | 1.8 |
| Suspension | Aerobic | 15 3 | 83 8 | — | — | 21.4 | 78.3 | — | + |
| | Anaerobic | 19 7 | 77 9 | + | 2 0 | 25 6 | 72 3 | + | 1 5 |

[a] Incubation time 3 hours.

[b] + = less than 1%

[c] O = Percent of $I^{131}$ immobile in chromatography.

different iodinated components on the paper strips was determined by measuring the area occupied by each $I^{131}$ peak.

Paper electrophoresis (Whatman No. 3) in barbital buffer at pH 8.6 or in 0.2 $M$ $(NH_4)_2CO_3$ buffer at pH 9.0 was also employed, to distinguish between iodothyronines and their alanine side-chain derivatives. The techniques have been described elsewhere (26, 28).

As inorganic iodide was found to be a consistent and, in most cases, the major iodinated metabolite after incubation, periodic batches of samples were analyzed for their radioiodide content by a method previously described (30). This was done by the oxidation of radioiodide (after the addition of 50–100 $\mu$g. of stable iodide and organic iodinated compounds as carriers) by $Fe^{+++}$ ions. The elemental iodine was extracted by $CS_2$ and absorbed in a $Na_2S_2O_3$ solution before measurement of $I^{131}$. Good agreement was found in the results obtained with the two different methods used for the estimation of the fraction of $I^{131}$ in the form of iodide.[4]

## II. RESULTS

### 1. Brain Slices and "Concentrated" Suspensions

In the preliminary experiments, the tissue concentration for both slices and homogenates was in the range of 1 g. wet weight/1–3 ml. of suspension medium. Substrate concentrations were varied from $10^{-5}$ $M$ to $5 \times 10^{-9}$ $M$. Table I summarizes the analytical data on dog brain incubation samples examined after three hours of incubation at concentrations of thyroxine and triiodothyronine much below the level of iodine found in the serum of the same animals. Normal dog serum iodine is about 2.5 $\mu$g % or, if it is all thyroxine, about $5 \times 10^{-8}$ $M$. Rat and human serum contains about 5 $\mu$g.% organic iodine or, if it is all thyroxine, a concentration of about $10^{-7}$ $M$.

Definite degradation of both the hormones is noticed in all the samples. The major portion of the radioactivity was transferred from the substrate to the fraction identified as inorganic iodide and less than 5% of $I^{131}$ could be detected in any other component outside of the unchanged thyroxine or 3 5 3'-triiodothyronine. Almost the same pattern of $I^{131}$ distribution is found irrespective of whether the tissue was homogenized or sliced and under aerobic or anaerobic conditions. The presence of either glucose or $PO_4$ did not alter the results. Similar results of de-

[4] We are very grateful to the members of the staff of Warner-Chilcott Laboratories, Morris Plains, New Jersey, for having supplied us with all the derivatives of the alanine side-chain of thyroxine and L-3 5 3'-triiodothyronine used in this work.

TABLE II

DEGRADATION OF L-THYROXINE AND L-3,5,3′-TRIIODOTHYRONINE IN "DILUTE" SUSPENSION (1 G TISSUE/15 ML MEDIUM) OF DOG AND RAT BRAIN

| Species | Hours of incubation | L-Thyroxine, 1.6 × $10^{-9}$ *M* % Total $I^{131}$ distributed as | | | | L-Triiodothyronine, 1.8 × $10^{-9}$ *M* % Total $I^{131}$ distributed as | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_4$ | $I^-$ | O | Unidentified | $T_3$ | $I^-$ | O | Unidentified |
| Dog | 1 | 58.0 | 23.3 | — | 18.7 | 69.2 | 22.0 | 1.5 | 6.9 |
| | 3 | 45.6 | 41.0 | + | 9.0 | 55.2 | 39.1 | — | 5.7 |
| | 6 | 41.0 | 54.7 | 1.0 | 3.3 | 54.4 | 42.6 | 2.0 | 1.2 |
| Rat | 1 | 63.7 | 16.6 | 2.3 | 17.4 | 73.5 | 14.2 | — | 12.8 |
| | 3 | 58.8 | 28.0 | + | 13.2 | 67.1 | 22.5 | 3.0 | 7.4 |
| | 6 | 46.3 | 51.8 | — | 1.8 | 60.0 | 39.8 | + | — |
| Controls | 6 | 88.4 | 10.7 | + | — | 90.9 | 8.1 | 1.0 | — |

iodination were obtained with both rat and chick brain preparations In all cases the rate and extent of degradation were considerably diminished near concentrations of $5 \times 10^{6}$ to $10^{5}$ *M* thyroxine or 3:5:3'-triiodothyronine.

## 2. "Dilute" Suspensions

It was hoped that a dilution of the tissue system would lead to a retardation in the formation of iodide and detection of possible short-lived intermediate substances. As no significant differences were found between the different samples in more concentrated systems, only results with tissue suspensions will be described. The most usual tissue concentration used was 1 g. wet weight/15 ml. of suspension medium. Analytical data obtained at one, three, and six hours after incubation using "dilute" dog and rat brain suspensions are compared in Table II.

Two effects of dilution of the tissue system are of interest: (a) A reduction in the rate of degradation of the substrate by a factor of over three, (b) the consistent occurrence of a substantial fraction of total $I^{131}$ in chromatogram peaks described as "unidentified." This fraction was found to consist of more than one iodinated component and will be discussed later. In all cases, the amount of $I^{131}$ in this unknown fraction reached a peak in the early time intervals and decreased very markedly after the first hour of incubation. In experiments with thyroxine, a small amount of $I^{131}$ (never exceeding 5–8% of the total) was found in the peak corresponding to 3.5·3'-triiodothyronine The occurrence of $T_3$ was, however, not a regular feature and this compound has been included in the group of unidentified substances mentioned in Table II

To determine something of the nature of what is presumed to be one or more enzymes responsible for the degradation of thyroxine and triiodothyronine, certain well-known inhibitors of enzymes and of biological oxidation were studied (43) The results are presented in Table III

Only two substances produced significant inhibitions in the rate and nature of degradation of thyroxine and 3.5 3'-triiodothyronine, 2-octanol and $HgCl_2$ The latter substance is of especial interest as it effectively prevented the formation of any inorganic iodide after incubation, while permitting the formation of large amounts of unidentified iodinated compounds Furthermore, with the precipitation of $HgCl_2$ with $H_2S$ and reincubation for six hours, the inhibition was reversed with the formation of the usual amount of iodide and a rapid diminution in the amount of the other iodinated products. $NaAsO_2$ accelerated the rate of breakdown of thyroxine only. But for both the hormones, iodide was the only iodinated metabolite found after incubation with $NaAsO_2$

### 3 Time-Curve Studies and Effect of Substrate Concentration

Although the fraction of total $I^{131}$ in all the major iodinated compounds was determined in all cases, only the values of $I^{131}$ in the unchanged thyroxine or 3.5·3′-triiodothyronine were considered for time-curve studies In earlier experiments the substrate concentration was found to be a major factor determining the results obtained and this problem was also studied further. "Dilute" suspensions were used for all quantitative studies and some typical results on dog, rat, and chick brain preparations are presented in Fig 1

A very similar pattern is observed in the way both thyroxine and 3 5:3′-triiodothyronine are metabolized by the preparations of dog, rat, and chick brains. The slope of the degradation curve which is steep for the first 2–3 hours flattens out considerably by the sixth hour, and at lower levels of substrate concentration, it shows a multiphase character. A high degree of sensitivity of the rate of degradation to the amount of substrate present is observed. At doses near the serum level of thyroxine, i e., $5 \times 10^{-8}$ $M$ to $10^{-7}$ $M$, a very near total inhibition of the whole system is brought about. In more concentrated brain suspension the threshold for this inhibition due to substrate concentration is raised more or less proportionately. For example, the following result was obtained with rat brain suspensions at 1 g. tissue/15 0 ml. medium, six hours after incubation with thyroxine

| | | | | |
|---|---|---|---|---|
| Substrate concentration | $5 \times 10^{-8}$ $M$ | $10^{-7}$ $M$ | $5 \times 10^{-7}$ $M$ | $5 \times 10^{-6}$ $M$ |
| Per cent total $I^{131}$ as unchanged thyroxine | 41 2 | 62 7 | 69 5 | 84 4 |

In view of the greater biological and chemical instability of 3.5.3′-triiodothyronine (15, 43) it was surprising to note that thyroxine was consistently degraded at a more rapid rate than 3 5 3′-triiodothyronine.

### 4. The Nature of Metabolites of L-Thyroxine and L-3 5 3′-Triiodothyronine

Inorganic iodide was the major and most consistent iodinated metabolite of all incubations. The group of iodinated compounds designated so far as "unidentified" were also found consistently in all experiments performed with "dilute" systems, it has also been seen that quantitatively their occurrence followed a regular and definite pattern Attempts made to identify these iodinated substances are described below.

In Fig. 2 are reproduced some typical chromatographic recordings of $I^{131}$ distribution in samples of "dilute" brain tissue suspensions after in-

TABLE III

DEGRADATION OF L-THYROXINE AND L-3 5 3'-TRIIODOTHYRONINE IN "DILUTE" RAT BRAIN SUSPENSION IN THE PRESENCE OF VARIOUS SUBSTANCES[a]

| Substance added | Concentration | L-Thyroxine, $1.8 \times 10^{-9}$ M % Total $I^{131}$ distributed as | | | | L-Triiodothyronine, $1.8 \times 10^{-9}$ M % Total $I^{131}$ distributed as | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_4$ | $I^-$ | O | Unidentified | $T_3$ | $I^-$ | O | Unidentified |
| None | | 48.2 | 46.6 | — | 5.2 | 55.3 | 37.5 | — | 6.2 |
| Control | | 88.9 | 11.1 | — | — | 89.9 | 10.1 | — | — |
| Na CN | $10^{-3}$ M | 73.7 | 23.3 | — | 3.0 | 66.0 | 31.0 | 2.1 | + |
| Na $AsO_2$ | $10^{-3}$ M | 30.2 | 69.8 | — | — | 57.3 | 42.6 | — | — |
| Na F | $10^{-2}$ M | 66.0 | 29.0 | + | 4.8 | 63.7 | 29.8 | 3.0 | 3.5 |
| Octan-2-Ol | Saturated solution | 58.2 | 22.9 | — | 8.7 | 71.6 | 22.0 | + | 6.0 |
| Methylene blue | 0.05% (w/v) | 58.2 | 35.8 | 2.5 | 3.5 | 52.1 | 47.6 | — | + |
| Janus green | 0.005% (w/v) | 67.6 | 28.1 | + | 4.0 | 56.3 | 39.3 | + | 5.1 |
| Hg $Cl_2$ (before $H_2S$) | $10^{-3}$ M | 68.6 | 6.1 | 4.0 | 21.3 | 75.0 | 5.2 | 1.8 | 18.0 |
| Hg $Cl_2$ (after $H_2S$) | $10^{-3}$ M | 39.2 | 51.4 | 3.0 | 5.3 | 27.0 | 63.1 | 4.1 | 4.1 |
| $H_2S$ alone | Saturated | 41.5 | 54.7 | 2.0 | 1.5 | 46.1 | 51.0 | — | 2.5 |

[a] Incubation time 6 hours

the paper corresponding to the different $I^{131}$ components were eluted with *n*-butanol followed by 5% $NH_4OH$. The eluates were concentrated under reduced pressure (5–12 mm.Hg) at temperatures below 25°C. and

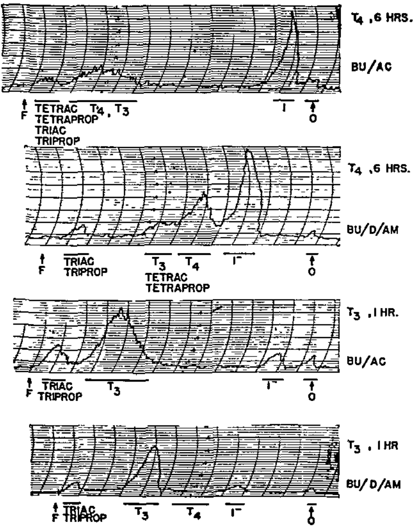


FIG 2 $I^{131}$ recording of chromatograms of iodinated products of incubation of L-thyroxine ($T_4$) and L-3 5·3'-triiodothyronine ($T_3$) with "dilute" rat brain suspension (1 gm tissue/15 ml. suspension medium) Solvent systems Bu/Ac = *n*-butanol-acetic acid-water, Bu/D/Am = *n*-butanol-dioxane-ammonia, O = origin of chromatogram, F = solvent fronts

cubation with thyroxine (for 6 hours) or 3:5:3′-triiodothyronine (for 1 hour). The distribution and homogeneity of the unidentified components depends upon the chromatographic solvent system used.

In order to be satisfied that the different chromatographic peaks were not artifacts, 25-cm.-wide preparative chromatograms were made with 0.2 to 0.4 ml. of each incubation sample After development, portions of

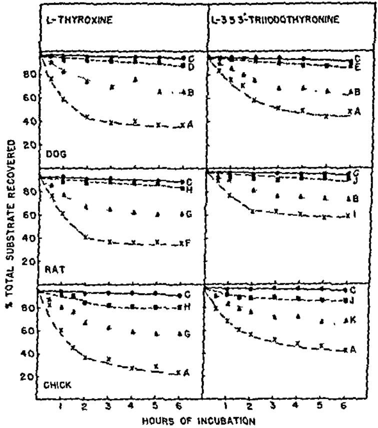


FIG. 1. Degradation of L-thyroxine ($T_4$) and L-3 5,3′-triiodothyronine ($T_3$) when incubated with "dilute" dog, rat, and chick brain suspensions (*1 gm tissue/15 ml. suspension medium*) and the effect of substrate concentration on the rate of degradation, in function of time. Each value represents the average from two independent incubations analyzed by not less than two different solvent systems for chromatography. Substrate concentrations $A = 5 \times 10^{-9}$ *M*, $B = 2 \times 10^{-8}$ *M*, $D = 1.3 \times 10^{-6}$ *M*, $E = 10^{-6}$ *M*, $F = 3.5 \times 10^{-9}$ *M*, $G = 4 \times 10^{-8}$ *M*, $H = 6 \times 10^{-7}$ *M*, $I = 4 \times 10^{-9}$ *M*, $J = 7 \times 10^{-7}$ *M*, $K = 5 \times 10^{-8}$ *M*
C = Control incubations

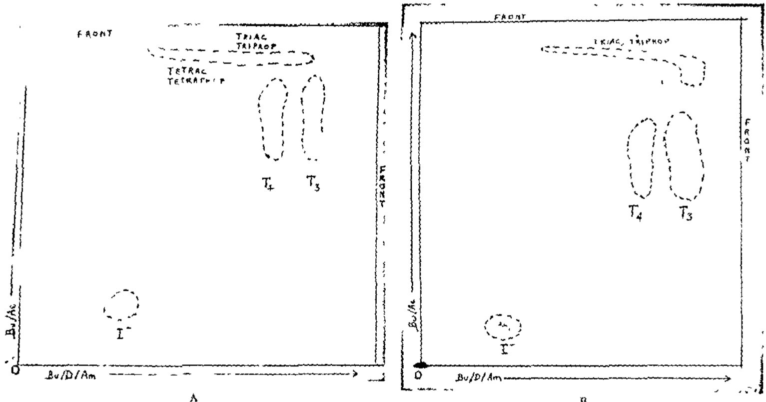


Fig. 3 Radioautogram of two-dimensional chromatography of iodinated products of incubation of A L-thyroxine ($T_4$) and B L-3 5 3'-triiodothyronine ($T_3$) with 'dilute' rat brain suspension (1 gm tissue/15 ml suspension medium) Abbreviations refer to position of added carriers Dotted lines represent the position of different carriers as revealed by chemical staining Solvent systems Bu/Ac = n-butanol-acetic acid-water Bu D Am = n-butanol-dioxane-ammonia, O = origin of chromatogram

rechromatographed with the four different solvent systems. In each case, not less than 85% of the radioactivity was recovered in a single peak with an $R_f$ value in the same solvent system that was identical to that in the original preparative chromatogram.

Hence for thyroxine, the non-iodide iodinated component near the solvent front was found in one peak when the chromatographic solvent system was *n*-butanol-acetic acid, but this was resolved into two peaks when the solvent system used was *n*-butanol-dioxane-ammonia, or ter-amyl alcohol-ammonia. For triiodothyronine only one iodinated peak, outside of iodide, was detected with all the four different chromatographic solvent systems. A comparison of the $R_f$ values of the carrier substances added showed that chromatographic properties of the following compounds were identical to the two "unidentified" iodinated compounds obtained by the incubation of thyroxine: TETRAC, TETRAPROP, TRIAC, TRIPROP, and $T_3$ With triiodothyronine, this could be TRIAC or TRIPROP. TRIFORM and TETRAFORM which appeared also as likely metabolites from chromatographic studies were eliminated from the results of electrophoretic analysis.

In bidimensional chromatograms (Fig. 3) developed with *n*-butanol-acetic-acid-$H_2O$ and *n*-butanol-dioxane systems, the triiodo and tetraiodo groups of side-chain analogs are not as well resolved as would be expected from results in each of the single dimensions (possibly due to employing larger amounts of the sample than in monodimensional chromatograms). Yet in all cases, the spots on the autograms of bidimensional chromatograms were exactly superimposable on the chemically stained spots of the carriers added. In the figure the dotted lines actually represent the tracing of the area occupied by the various carrier substances added before development of the chromatograms The faint spot of $T_3$ observed on the chromatogram of a $T_4$ incubation (Fig. 3a) was detected in less than half the samples studied whereas the other metabolites were consistently present.

The possibility that the group of iodinated compounds found besides the iodothyronines and iodide represent the acetic and propionic acid analogs of $T_4$ and $T_3$, was strengthened by results obtained with paper electrophoretic analysis (Fig 4).

The iodothyronines were almost immobile under the conditions employed while their side-chain analogs migrate 3–5 cm. toward the anode in 6 hours. Iodide migrated at a very rapid rate and is not shown in Fig. 4. The percentage distribution of $I^{131}$ in electrophoretic analysis was compatible with figures obtained from chromatographic analysis

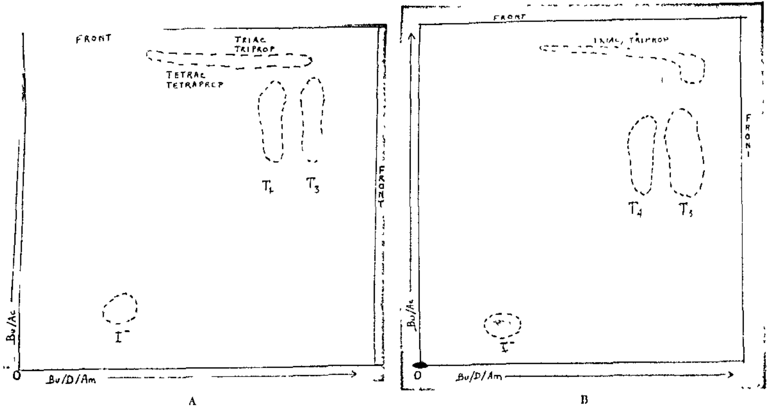


FIG 3 Radioautogram of two-dimensional chromatography of iodinated products of incubation of A L-thyroxine ($T_4$) and B L-3,5,3′-triiodothyronine ($T_3$) with "dilute" rat brain suspension (1 gm tissue/15 ml suspension medium) Abbreviations refer to position of added carriers Dotted lines represent the position of different carriers as revealed by chemical staining. Solvent systems Bu/Ac = *n*-butanol-acetic acid-water; Bu/D/Am = *n*-butanol-dioxane-ammonia, O = origin of chromatogram

The chromatographic characteristics of any of the unknown iodinated compounds were not found to correspond to the triiodo- and tetraiodothyropyruvic acids (33), thyroxamine (41), o-methyl-thyroxine or the glucorinic acid conjugates of thyroxine and triiodothyronine (31). Attempts to detect 3.3′-diiodothyronine as one of the metabolites were unsuccessful as this compound was found to migrate poorly in the methanol-$CH_3COONH_4$ system (32) in the presence of brain tissue

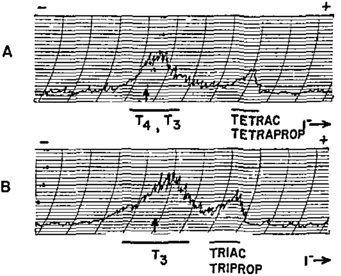


FIG 4 Paper electrophoresis of iodinated products of incubation of L-thyroxine and L-3 5 3′-triiodothyronine with "dilute" rat brain suspension (1 gram tissue/15 ml suspension medium) Electrophoresis with 0 2 *M* $(NH_4)_2CO_3$ buffer at pH 9 1 at 135 volts for 6 hours Position of inorganic iodide not shown

suspension With the use of analytical techniques described here, it was only possible to separate the acetic and propionic acid analogs of the two hormones in each group of the triiodinated and tetraiodinated derivatives, i e, it was not possible to determine whether the alanine side-chain of $T_4$ and $T_3$ was degraded to two or three carbon atoms

## III DISCUSSION

The results just described show that there are in dog, rat, and chick brain, systems, presumably enzymatic, which degrade thyroxine and 3.5.3′-triiodothyronine The active system in the tissue is thermolabile, being inactivated by heating at 100° for 20–30 minutes and its activity exhibits little variation under aerobic or anaerobic conditions The tissue

structure need not be preserved for this activity since cell-free preparations were obtained that possessed properties identical to those observed in brain tissue slices and cell suspensions[5]

Prior to this work, Sprott and Maclagan (37) had described a system in rat liver, and other tissues (including brain), which was said to deiodinate thyroxine and triiodothyronine. Since this system was barely active at a physiological pH, inhibited by cyanide and anaerobiosis, and difficult to inactivate by boiling, it is not readily comparable to the brain system here described. Furthermore, their tissue preparation appeared to be active at substrate concentrations of 20 to 25,000 times the level of thyroxine in blood, whereas the system just described is almost completely inactive at these levels. The difficulty in comparing our results with those of Sprott and Maclagan is enhanced by the differences in techniques used to determine deiodination or degradation of the hormones Larson *et al.* (21) have described an "adaptive thyroxine deiodinating system" in rat kidney slices which converts thyroxine to triiodothyronine. In our experience (see Fig. 2) the solvent systems for chromatography used by these workers could not clearly differentiate between $T_3$, TETRAC, and TETRAPROP. More recently however, Albright *et al* (1) have reported the formation of TETRAC from thyroxine following incubation with rat kidney mitochondria

It is important to note in considering this work that only the iodine atoms of the phenolic ring of the $T_3$ and $T_4$ molecules were labeled with $I^{131}$. Upon the loss of these iodine atoms, all track of the fate of the rest of the thyroid hormones is lost.

From the results described in the present work, the rate of degradation of the two hormones and the nature of the products formed were dependent principally on both the concentration of the tissue preparation and that of the substrate itself. With the "concentrated" systems (Table I), only a gross deiodination of thyroxine and triiodothyronine is observed The dilution of the preparations (Table II) not only led to a diminution in the rate degradation but iodinated compounds not observed in more concentrated preparations were detected in significant amounts, especially during the early time intervals. Prolonged incubations (from 6 to 12 hours) with the "dilute" preparations also resulted in finding inorganic iodide as the only major iodinated metabolite. It appears that more than one enzyme system is involved in the ultimate degradation of thyroxine and 3.5 3'-triiodothyronine of iodide and that dilution of the

[5] A subsequent communication will deal with the problem of localization and fractionation of the active component in brain tissue

preparations affects to a different extent the reaction velocities of each system (38). Hence, it is likely that the group of unidentified compounds were actually formed in the "concentrated" preparations but were too rapidly deiodinated to be observed. Different substrate dose levels used with "dilute" preparations varied from concentrations 10 to 20 times lower to those about 1,000 times higher than the circulating level of thyroxine Since the exact level of 3:5·3'-triiodothyronine in serum is not yet well known (7, 40), no comparison is possible. The unexpected slower rate of degradation of triiodothyronine than of thyroxine is difficult to explain. The high degree of sensitivity of the brain preparations to metabolize the substrates at increasing dose levels would reflect the absence of an efficient detoxication mechanism as found in the liver and to some extent in the kidney (31, 39).

The unknown iodinated compound observed by Alpers and Rall (2) in human cerebrospinal fluid was not encountered among the incubation products analyzed On the other hand, what chromatographically appears to be a series of derivatives of deamination of the alanine side-chain of thyroxine and 3 5 3'-triiodothyronine were characterized, along with iodide. The significance of finding these derivatives arises from the fact that the pyruvic, propionic, acetic, and formic acid derivatives of thyroxine and 3:5:3'-triiodothyronine side-chain have been recently reported to have some properties of the thyroid hormones and, in some biological tests, they posess a higher potency or a shorter time lag preceding their action (9, 24, 25, 34, 35). Barker (5), however, has not been able to reproduce some of the biological activity tests on these derivatives Of these derivatives, the triiodo- and tetraiodo-pyruvic acid analogs have been found in bile and urine but in significant amounts only after the administration of high doses of the corresponding amino acids (33). More recently, Roche *et al.* (29) have identified TRIAC in kidneys of thyroidectomized rats injected with triiodothyronine. It should be pointed out here that in our work it was not possible to distinguish TRIAC from TRIPROP and similarly TETRAC from TETRAPROP by using the available methods of paper chromatography or electrophoresis (Figs. 2, 3, and 4) Hence, it is not clear at what step the side-chain degradation ceases before the molecule loses its iodine atoms in the 3' and 5' positions Thyroxine can also undergo a direct partial deiodination with any side-chain modification to give 3:5·3'-triiodothyronine, although the amount and frequency of occurrence of the latter substance were low. Direct deiodination of 3.5 3'-triiodothyronine was not observed, unlike the simultaneous formation of 3 3'-diiodothyronine and

iodide with TRIAC in rat kidney after the administration of 3.5.3′-triodothyronine (29).

The reversible inhibition of deiodination of the substrates by $HgCl_2$ strongly supports the hypothesis that there are at least two enzyme systems responsible for degradation of thyroxine and triiodothyronine. Here the inhibitor appears to block an enzyme system capable of deiodinating only the side-chain derivatives of thyroxine and 3.5·3′-triiodothyronine, while exerting no action on the system responsible for the deamination of the hormones It also suggests that the principal fashion of deiodination of thyroxine and 3.5:3′-triiodothyronine by the brain tissue is through a deamination of their alanine side-chain.

No evidence is as yet available as to whether the transformations of L-thyroxine and L-3:5:3′-triiodothyronine observed *in vitro*, are reproduced in the central nervous system of intact animals, although work has been begun on this problem It is possible that at the cellular level the thyroid hormones undergo a series of chemical transformations in exerting their biological action and therefore the identification of their metabolites reflect in part a mode of action Although this is a tempting conclusion, this work only opens the question of whether the transformations here observed are designed to provide the central nervous system with fast-acting biologically active metabolites of the thyroid hormones or whether they just represent a chain of metabolic reactions commonly undergone by aromatic amino acids (23).

## IV. SUMMARY

Rat, chick, and dog brain tissue possess systems, presumably enzymatic, capable of deaminating and deiodinating thyroxine and 3 5.3′-triiodothyronine. These systems are active at a physiological pH (pH = 7.45) and their activity is highly sensitive to changes in both the concentration of substrate and that of the tissue preparation itself. These systems are unaffected by cyanide or anaerobiosis but inactivated by heating at 100°C Mercuric chloride reversibly inhibits the deiodinating mechanism while the deaminating mechanism is unaffected Under these conditions or with dilute preparations of the brain tissue, the propionic and/or acetic acid analogs of both thyroxine and triiodothyronine are formed. It was not possible to distinguish TETRAC from TETRAPROP and similarly TRIAC from TRIPROP but one or more of these were formed. The major product of deiodination was iodide but in some cases a small amount of triiodothyronine was formed as a product of incubation of thyroxine.

### Acknowledgments

Sincere thanks are due to Doctors J. E. Rall, R. W. Rawson, and W. L. Money for their assistance and very helpful suggestions.

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## Discussion

G. Pincus Did you imply in your last statement that these deaminated derivatives act more rapidly than the amines?

J R. Tata. Than the amino acids This has been a controversial point There are workers who maintain very categorically that there is no such action and there are others who do support it When I say "biological action" I go beyond the conventional measurements of oxygen consumption. For example, the acetic acid analog of triiodothyronine does not bring about a significant increase in oxygen consumption in humans, but will bring about rapid lowering of cholesterol and an increase in the nitrogen and phosphorus in the urine In this respect it acts faster than triiodothyronine. When I say "action" it is not oxygen consumption, but it could mean some other action which we have not been able to measure directly

B B Brodie I enjoyed your paper very much I was wondering about the specificity of your analytical method Did you measure $C^{14}$ counts or actually thyroxine?

J R Tata Actually thyroxine The tissue was analyzed by chromatography

B B Brodie For my own information, what evidence is there that the triiodo compound is more active than the thyroxine?

J R Tata: So far in all biological activity measurements, the serum cholesterol level test, the goiter-prevention action are consistent, about 3 to 10 times more active than the tetraiodinated analog

B B Brodie Does dehalogenation occur in tissue homogenates?

J. R. TATA: For dehalogenation, most of our work has been with the homogenates I would like to point out that we have now given up working with homogenates or slices. We have pure cell-free preparations where you can reproduce exactly what you see with the crude preparations and, using the mercuric chloride inhibition of deiodination, we hope to purify the mechanism of dehalogenation. We believe it is an enzyme which could be reversibly blocked by mercuric chloride.

B. B. BRODIE: Have you been able to identify a coenzyme?

J R TATA: No coenzyme, but the cell preparation loses activity on dialysis It loses activity when dialyzed against the same isotonic saline solution used for incubation.

S UDENFRIEND. As to mercuric chloride, what is the evidence that mercuric chloride is acting at the enzyme level, and not at the substrate level? Mercury can readily form complexes.

J. R. TATA: We have done experiments by using just the mercuric chloride and thyroxine alone without any enzyme from the tissue.

S. UDENFRIEND: Is it possible that what you are doing is making a complex of mercury and thyroxine, which is no longer acted on by the enzyme?

J R TATA Yes, but you do get transformation of thyroxine into the side-chain analogs. I cannot say this very definitely, but we feel it is blocking the protein enzyme because we have isolated a mercuric chloride-complexed protein and we could dissociate the mercuric chloride, and this enzyme will deiodinate thyroxine.

H J. KOCH, JR I had a question about cadmium chloride—whether it showed the same inhibition as mercuric chloride

J R. TATA. We have not tried cadmium chloride

C G HARTMAN You don't give much credence to cholesterol level depression in your tests Is the cholesterol level a pretty poor sort of test?

R W. RAWSON: In human myxedema the serum cholesterol is a good yardstick, not only as a diagnostic tool, but also for evaluating the effects of therapy.

G PINCUS Is there very good evidence for deaminated compounds having a very specific effect on the central nervous system?

J. R TATA I don't know of any The first evidence that this might not be a specific effect was provided by Kharasch and his associates in that the propionic acid analog of thyroxine was more active than thyroxine itself, and this difference was about 150-fold or 200-fold You required 200 times less of propionic acid analogs to get the same effect

# Decreased Appetite for Alcohol and Alcoholic Beverages Produced in Rats by Thyroid Treatment[1]

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Dietary self-selection studies have demonstrated the ability of rats to make beneficial dietary selections from a great variety of substances—nutritional, harmless, and poisonous—and under a variety of conditions (2). Appetite was demonstrated to be such a reliable guide to dietary needs of the rat that it can be used to determine whether a substance has a nutritive value or is harmful It has been used to determine the nutritive value of a number of carbohydrates, fats, proteins, and minerals, as well as of whole foods. Since in general the dietary needs of rats and man, and the functioning of their entire gustatory system parallel one another so closely, appetite of rats can also be used to throw light on the possible food values of various substances for man.

The self-selection method has also been applied to alcohol. These studies concern the following questions.

1. Does the rat regard alcohol as a food or a poison?
2. If as a food, how much alcohol will it take voluntarily and in what concentrations?
3. How can a rat be made to take harmful amounts of alcohol, that is, to become addicted?
4. How can the rat be made to stop drinking alcohol?

Our early studies demonstrated that rats use alcohol as food (1, 4). Thus, when fluid intake was restricted to an 8, 16, or 24% solution of alcohol, the rats reduced their food intake in direct proportion to the calories received from alcohol. In some instances the alcohol intake con-

[1] Carried out under a grant from the Division of Research Grants and Fellowships of the National Institutes of Health and the Johns Hopkins University and under a grant from the Committee on Problems of Alcohol, National Research Council, to the Johns Hopkins University

Thyroid powder was supplied by the Armour Laboratories, Chicago, Illinois; the thyroxine and triiodothyronine were supplied by Smith, Kline and French, Philadelphia, Pennsylvania.

stituted 40–50% of the total caloric intake. On this high alcohol intake the rats grew at a normal rate and were normal in all respects. Some were observed as long as 15 months.

In other experiments it was demonstrated that rats preferred alcohol solutions to water in concentrations up to 7–8% (1). They drank fair amounts of alcohol in concentrations of 10% but only small amounts of higher concentrations. There were of course considerable individual variations in the amounts of alcohol that the rats drank.

So far we have not succeeded in addicting ordinary tame laboratory rats to alcohol by any method. We have, however, succeeded in addicting two fierce, aggressive wild rats trapped from the streets. This was done by restricting fluid intake to a 20% solution of alcohol over a period of 6 months. When given a choice of food, water, and a 20% solution of alcohol, these rats gradually ate less food, drank less plain water, and more alcohol. As a result they progressively lost weight and finally died.

More recently we have been interested in reducing voluntary intake of alcohol rather than in increasing it.

In these experiments the rats had access to our stock diet in a non-spillable food cup and to two graduated inverted 100-ml. drinking fountains, one filled with water, the other with a 10% alcohol solution.

Records were made daily of the intake of food and fluids and weekly of body weight To obtain base lines records were taken for at least 30 days before the start of any form of treatment.

A total of 72 domesticated Norway rats from our colony were used. The ages of the rats ranged from 100 to 270 days.

In the first experiments the rats were treated with thyroid extract. How we happened to use thyroid need not be told here. It should suffice to say that it was not on any logical basis.

Thyroid powder was mixed with the stock diet. The concentrations used ranged from 1% to 0 02%

Figure 1 shows a typical record of a rat that received the 1% concentration. The ordinates give the intake of fluids in milliliters, food intake, and body weight in grams; the abscissas, the age in days The arrow marks the start of the thyroid diet. On the stock diet the intake of alcohol remained fairly constant near 20 ml, water near 3 ml. and the intake of food near 10 grams. Almost immediately after the start of the diet this rat began to drink less alcohol and more water and to eat more food. After 25 days it practically refused alcohol.

A 0.1% concentration of thyroid powder reduced the alcohol intake

but more slowly; a 0 04% (4 mg. per day) even more slowly, and a 0.02% had no effect.

Thyroxine (0 2 mg per day) and triiodothyronme (50 $\mu$g ) also reduced the alcohol to low levels.

Thyroid feeding (0 04%) greatly reduced or eliminated the intake of the alcoholic beverages—wine (ordinary table wine) and whisky (di-

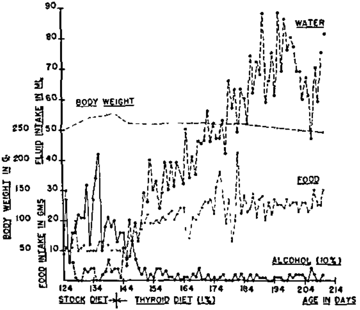


FIG. 1. Graph showing the daily intake of food, water, and a 10% alcohol solution before and after treatment with thyroid powder (1% of stock diet).

luted to 10%), it reduced the intake of beer but only to a slightly lower level The difference between the effects on appetite for alcohol, wine, or whisky on the one hand, and for beer on the other, may be accounted for by the carbohydrate content of beer since earlier experiments showed that thyroid feeding greatly increases the appetite for all of the common sugars, especially maltose, dextrose, sucrose, and fructose (3). Beer appetite would thus be the resultant of the appetites for the two substances

Preliminary experiments showed that rats made hypothyroid by surgical removal of the thyroid or by treatment with $I^{131}$ may have an increased appetite for alcohol. So far the $I^{131}$ rats have shown the greatest increase in alcohol intake.

Here it is of interest that some of the $I^{131}$-treated rats showed abnormal but regular cycles in running activity, food, and water intake and in body weight—ranging from 56 to 18 days (5). These rats are now being tested to determine whether their appetite for alcohol shows corresponding cyclical changes.

That hyperthyroid rats refuse alcohol solutions must indicate that in their condition they are not able to use alcohol, that ingestion of alcohol may be harmful to them. Actually we have found that forced ingestion of alcohol solutions produced marked pathological effect on the adrenals, liver, and kidneys of hyperthyroid rats.

To what extent can these results be applied to man? Attention has already been called to the close relationship between the appetite shown by rats and man for various nutritional or poisonous substances. The results would thus indicate that an increased appetite for alcohol might indicate the presence of a hypothyroid condition and that treatment with thyroid might reduce the abnormal appetite for alcohol. Spree drinking might then reflect the cyclical changes in metabolism that were produced experimentally in rats by reducing thyroid function.

Caution in the application of the results to man must be exercised since an alcoholic under treatment with thyroid who, under social pressure or other forces, still persists in drinking large amounts of alcohol, might do himself damage. It may be mentioned, however, that in the few instances in which thyroid treatment has been tried it has greatly reduced the appetite for alcohol and at the same time greatly increased the appetite for sugars and food and for plain water, just as it does in rats

In summary, it was found that treatment of rats with thyroid preparations reduced appetite for alcohol and increased appetite for food and thirst for water; thyroidectomy and treatment with $I^{131}$ had the opposite effect.*

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* Dr. Richter was unable to attend the conference There was thus no discussion of his paper —ED.

# The Thyroid Hormones and Their Relationships to Mental Health

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The first suggestion of a possible relationship between thyroid disease and mental disease is found in the philosophical writings of Paracelsus (19) whose works were published in 1603, 62 years after the death of this colorful and unorthodox student of medicine. In an heretical fashion he discussed the misfortunes of what he called fools, simpletons, or simple unknowing men who also had goiters Felix Platter (20) described the clinical picture of goiterous cretinism very accurately in his textbook of medicine which was first published in 1602 His description of cretinism was described under the heading "simple-mindedness, silliness and infantilism." He stated that "with propriety this diagnosis applied to those who are truly born simple, for in these infants we see the indications of simple-mindedness soon after birth. They neither learn to speak sensibly, much less to attend to other duties in which any industry is required" Curling in 1850 (7) reported for the first time the absence of thyroid tissue in two typically cretinous children and thus suggested thyroid insufficiency as the cause of this clinical picture.

In 1873, Gull (9) reported before the Clinical Society of London "on a cretinoid state supervening in adult life in women" what we now recognize under the name of myxedema. Gull described the personality of one of his patients as follows. "The mind which had previously been active and inquisitive, assumed a gentle, placid, indifference corresponding to the muscular languor, but the intellect was unimpaired." Even in the modern literature this is a common description of the myxedematous patient whom we think of as a placid, slow but pleasant and "gemütlich" individual.

However, if we refer to the report of the special committee on myxedema appointed by the Clinical Society of London (5), we find that untreated patients with this malady, such as that committee studied, may present various intellectual, temperamental, and psychiatric changes as part of their profound hypothyroidism. They reported "for a rather large proportion there is a more or less imperfection of mental processes, the

defect being one of retardation or sluggishness." "Irritability is a marked feature, though in exceptional cases the reverse is stated." "In some cases placidity alternates with occasional outbursts of fretfulness and irritability." "Delusions and hallucinations occur in nearly half the cases mainly where the disease is advanced. Insanity, as a complication, is noted in about the same proportion as delusions and hallucinations. It takes the form of acute or chronic mania, dementia and melancholia with a marked predominance of suspicion and self-accusation."

In the modern literature there are several reports of myxedematous patients who had been diagnosed as psychotic and recovered from their psychoses upon adequate replacement therapy with thyroid. Zondek and Wolfsohn (30) reported in 1944 their observations on a 23-year-old woman with myxedema of 18 months duration who presented changes characteristic of schizophrenia. The administration of large doses of thyroid resulted in a recovery from the psychosis which was evident on the fourth day and complete within a fortnight. Since the mental improvement was evident shortly after the characteristic diuresis but prior to any increase in the metabolic rate, they suggested that these changes were due to alterations in water and salt metabolism. In 1949 Asher (2) reported on 14 patients who had been admitted to a psychiatric hospital for various psychoses and who were found to have myxedema. Most of these patients had delusions of persecution and hallucinations. Some presented a picture of dementia, some were maniacal and others had depressions. Miller (18) reported in 1952 two patients with myxedema who were admitted to a psychiatric hospital, one because of a manic depression and the other with an acute depression. Both of these patients were rehabilitated as they were restored to a euthyroid state.

It has been reported that the electroencephalograms of myxedematous patients revealed a diminished voltage, absent alpha waves, and a slow frequency (3). Scheinberg and associates (26) have reported that myxedematous patients have more than a 30% decrease in cerebral blood flow and just less than a 30% decrease in cerebral oxygen and glucose consumption.

During the past nine years we have been studying the effect of total thyroidectomy with its resultant hypothyroidism, on the functional capacity of metastatic cancers of the thyroid Of 63 patients subjected to ablation of the thyroid we have observed the psychoses of myxedema in four as they were developing myxedema. One of these patients presented primarily a depression with periods of agitation The others had hallucinations and delusions of persecution. In one the hallucinations

were auditory. One of these patients was also paranoid. One of the patients died of her cancer before we instituted treatment with thyroid. The others recovered on restoring them to a euthyroid state.

We have not undertaken any systematic psychological investigation of our patients subjected to total thyroidectomy. However, Dr. Martha Schon, who is working under the direction of Dr. Arthur Sutherland at our Institution is investigating the psychological changes in patients with breast cancer who are being subjected to hypophysectomy—before removal of the pituitary, seven to eight weeks after hypophysectomy, while receiving cortisone in maintenance doses when clinical and chemical signs of myxedema usually develop, and again after restoration to a euthyroid state. Dr. Schon (27) has permitted me to quote some of her preliminary observations:

"The patients complained of tiredness. Most of the patients who were housewives reported that when myxedematous they were unable to carry on with their housework for any extended period of time. They admitted to good intentions to perform their work as prior to surgery, but stated that they were not able to complete a task. Others admitted that they were aware of accumulating dust and disorderliness in the home but were unable to do anything about it though this state of affairs irritated them considerably. They stated that they sought refuge in their beds where they felt most comfortable"

"Many patients complained that they did not sleep well, however, many times when the examiner visited them on the ward, they were asleep. When this was discussed later they either denied that they had slept or they were not aware of the length of time that they had slept. In some instances the patients fell asleep during a testing situation. They complained that no love or attention was extended to them. The patients while myxedematous were overcritical either of the hospital personnel or of their home environments. Whatever was done for them did not seem right. One patient reported that she could not tolerate the way her daughter arranged the linen or the dishes in the closet and that she would get into an argument with her because of it. When she was asked, several weeks later at a time when she was euthyroid, how she felt about that aspect of her home life, she related that everything seemed to be all right. Argumentativeness has also been noted, in most of these patients. Two patients reported that they had difficulties with their husbands with whom they had lived in perfect harmony for many years"

"Many patients complained that they could not stay with a task for a prolonged period of time. This was also reflected in their test-attitudes.

They became restless and gave up easily if the task required persistent effort and concentration. Frequently the testing had to be either interrupted for a time or had to be carried out on several occasions. On Rorschach testing the outstanding change between pre- and post-operative testing was decreased form-perception to the inkblots during the state of hypothyroidism."

Several years ago Dr. Hoskins (10, 11) and his associates undertook studies of thyroid function in patients with schizophrenia. They reported that the lowest of many basal metabolic rates in over 200 males with schizophrenia averaged 19% lower than the accepted standard. Brody and Mann (4), however, have reported that the protein-bound iodine levels in a large group of schizophrenic patients did not deviate significantly from the normal levels. Cranswick (6) has recently reported that a majority of 31 schizophrenic patients had greater thyroidal uptakes of $I^{131}$ than the majority of a comparable group of normals. Cranswick has also reported that the response of his patients to 10 mg. of thyrotropic hormone was less than that observed in his controls. Hoskins (11) has reported that about 10% of his patients with schizophrenia had clinical signs suggestive of hypothyroidism and such patients when treated with large doses of thyroid hormone experienced psychiatric improvement. One of the most interesting observations made by Hoskins is that these patients are quite resistant to large doses of thyroid hormone

Danziger and Knidwall (8) have recently reported a large series of patients with various psychiatric disorders that they had treated with thyroid in large doses and vitamins with or without conventional therapy Of 91 patients who recovered with such treatment, 28 relapsed within 6 to 72 months. They reported that such beneficial effects as were observed were obtained only after administering unusually large doses of thyroid for at least 100 days It is of interest that 26 of those who relapsed had stopped taking their thyroid 6 to 8 weeks prior to the relapse.

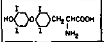
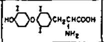
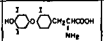
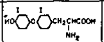
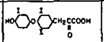
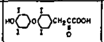
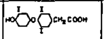
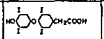
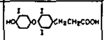
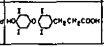
The observations by Hoskins of the low rates of oxygen consumption in schizophrenic patients when considered in the light of reports that such patients have normal serum protein bound iodines and normal thyroidal uptakes of $I^{131}$ raise several questions as to possible mechanisms

In the light of recent studies by Roche and his associates (25) an obvious study is that of determining the thyroid hormones synthesized by such patients. Roche and his associates have recently reported that they have isolated from the thyroids and sera of rats three iodinated thyronines other than thyroxine These compounds they have identified

as 3:5.3′-triiodothyronine, 3.3′.5′-triiodothyronine, and 3.3′-diiodothyronine. (See Table I and Fig. 1.) One of these compounds, 3.3′·5′-triiodothyronine, has much less biological activity than has thyroxine, while

TABLE I

THE THYROID HORMONES AND THEIR STRUCTURAL FORMULAE, AND THE TISSUES AND BODY FLUIDS IN WHICH THEY HAVE BEEN IDENTIFIED

| COMPOUND | STRUCTURAL FORMULA | THYROID | SERUM | BILE | TISSUES | URINE |
|---|---|---|---|---|---|---|
| Thyroxine |  | + | + | +[6] | + | + |
| 3 5 3-Triiodothyronine |  | + | + | +[6] | + | |
| 3 3 5′-Triiodothyronine |  | + | + | | | |
| 3 3-Diiodothyronine |  | + | + | +[6] | + | |
| 3 5 3-Triiodothyropyruvic Acid |  | | | + | | + |
| 3 5 3 5-Tetraiodothyropyruvic Acid |  | | | + | | + |
| 3 5 3-Triiodothyroacetic Acid |  | | | | + | |
| 3 5 3′ 5-Tetraiodothyroacetic Acid* |  | | | | | |
| 3 5 3-Triiodothyropropionic Acid |  | | | | +[7] | |
| 3 5 3 5′-Tetraiodothyropropionic Acid |  | | | | +[7] | |

3:5.3′-triiodothyronine is much more active than thyroxine. Of these iodinated compounds only thyroxine and 3:5:3′-triiodothyronine have been recovered from the serum of humans. It is quite likely, however, that the application of isotopic and chromatographic techniques such as those used by Roche and his associates will demonstrate either the same or similar iodinated compounds in the thyroid and sera of healthy

humans. The same techniques should be applied in determining what hormones are elaborated by the thyroids of schizophrenic patients.

If the normal protein-bound iodine levels that the schizophrenic patients are reported to have represent a normal distribution of thyroid hormones, one must also consider a possible abnormality in the disposition of the thyroid hormones. It has been demonstrated that thyroxine labeled with $I^{131}$ when administered to humans is disposed of in a fairly characteristic fashion. Most of it is excreted via the urinary tract.

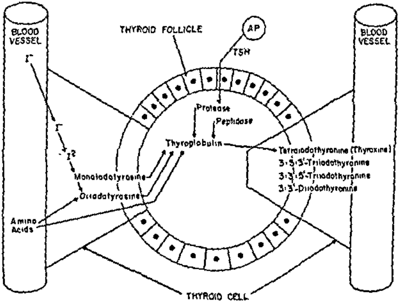



FIG. 1. Thyroid hormones which have been identified in thyroid and peripheral blood. This diagram illustrates present concepts concerning synthesis of thyroglobulin, its digestion by proteolytic enzymes under the influence of the anterior pituitary, and the thyroid hormones released

None of it is excreted as thyroxine; most of it is excreted as iodide. Ingbar *et al.* (12, 13) has recently reported that patients who have previously had Graves disease and even some of their siblings metabolize thyroxine differently than do normals. In our own laboratories we have found that the half-life in euthyroid athyreotic adults varies between 6 and 12 days. In one similar patient we have found that 3;5.3'-triiodothyronine had a half-life of 2½ days It might be fruitful to follow the fate of labeled thyroxine and its metabolic products in schizophrenic patients. In view of the observation by Alpers and Rall (1) of an as yet unidentified iodinated

compound in the spinal fluid of patients with Graves' disease and cancer of the thyroid, definitive studies of such iodinated compounds in the spinal fluid would seem most desirable. Every effort should be made to identify these compounds in the spinal fluid of normals. A natural step then would be to determine the capacity of the schizophrenic patient to convert thyroxine to such compounds. At the present time, because of limitations on the amount of radioactivity which can be given to patients with unlimited life expectancy, we are unable to administer to such patients labeled thyroxine with enough specific activity to permit definitive separation of the iodinated compounds recovered from the cerebrospinal fluid unless the patient has a life-limiting disease. It is hoped that the Atomic Energy Commission will permit such studies in patients whose lives, though not limited in years, are limited by the walls of our psychiatric institutions

Dr. Tata has reported to us this afternoon that slices or unwashed mitochondria of the cerebral cortex taken from chickens, rats, and dogs are capable of metabolizing labeled thyroxine to 3:5·3'-triiodothyronine and to tetraiodothyropropionic acid and/or tetraiodothyroacetic acid followed by deiodination to triiodothyropropionic acid and/or triiodothyroacetic acid and finally to iodide. If normal human brain tissue can be shown to metabolize thyroxine in a similar fashion, it would be most important to determine the capacity of brain tissue taken from schizophrenic and other psychotic patients to metabolize thyroxine. It is quite possible that the schizophrenic patient is relatively resistant to the thyroid hormone because his brain tissues are incapable of metabolizing thyroxine. In view of the observation described by Dr Tata it would be most desirable to compare the total metabolic effects of these various metabolites of thyroxine with those of thyroxine and other iodinated thyronines elaborated by the thyroid

In some of our assays of various thyroid hormones in human myxedema we have done metabolic balance studies and have followed the fate of nitrogen, phosphorus, calcium, sodium, chloride, potassium, and creatine as well as the changes in the rate of oxygen consumption and levels of serum cholesterol In one such study we have observed that one intravenous injection of triiodothyronine caused a prompt metabolic effect as contrasted with a slow response to a similar injection of thyroxine but did not differ either quantitatively or qualitatively from the net effect of thyroxine (23) (See Figs. 2a and 2b) Subsequent experiences in treating myxedema have demonstrated that the maintenance dose of triiodothyronine is one-third to one-quarter that of thyroxine.

In 1955 Lerman and Pitt-Rivers (16) reported that the administration of triiodothyroacetic acid to two myxedematous patients in daily doses up to 1 and 4 mg. failed to cause a rise in the basal metabolic rate but caused a disappearance of the myxedematous facies and a significant fall in the serum cholesterol. We (21) have observed that one 5-mg. dose of triiodothyroacetic acid when given intravenously to a myxedematous

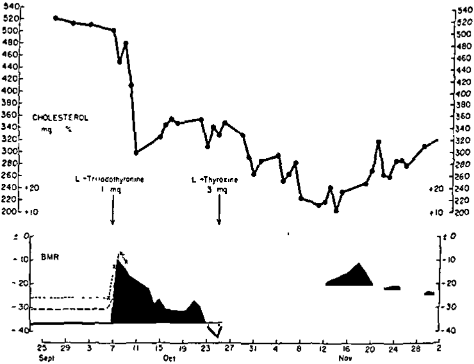



*Fig 2a* BMR *responses to 1 mg of* L-3 5 3'-*triiodothyronine and to 3 mg of* L-thyroxine, observed in an adult myxedematous female

patient failed to cause a significant increase in oxygen consumption. A 15-mg. dose of this agent did cause an increase in oxygen consumption. It is of considerable interest that the 5-mg dose of "triac" which did not cause an increase in oxygen consumption did cause a significant increase in urinary nitrogen, phosphorus, sodium, chloride, and potassium as well as a fall in serum cholesterol

One of the most interesting metabolic changes that we (22) have observed in our assays of the various thyroid hormones on myxedematous humans has been the marked urinary excretion of phosphorus. The ex-

creted phosphorus has in all instances been significantly greater than that amount of phosphorus which was calculated from the urinary nitrogen and calcium. Part but not all of this loss of phosphorus might be accounted for by a breakdown of phosphocreatine which is reflected by a marked increase in urinary creatine upon administration of any of these hormones. (See Figs. 3a and 3b.)

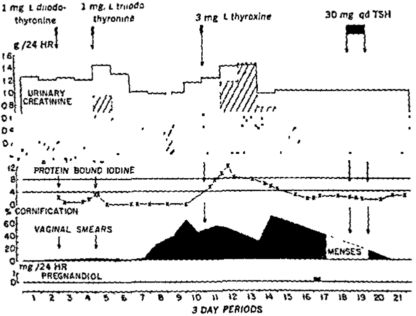



FIG 2b Excretion of creatine and creatinine by an adult myxedematous female after administering 1 mg of L-3·5 3'-triiodothyronine and 3 mg. of L-thyroxine.

Another explanation might be found in the thesis advanced by Lardy and Maley (15) who postulated that thyroxine may regulate metabolic rates by varying the efficiency with which cellular oxidations are coupled with phosphorylation. This theory is based on the foll [illegible] ... [illegible] form of high energy phosphate compounds, which are formed from inorganic phosphate. (b) The phosphate and high-energy phosphate compounds are involved in the scheme of electron transport. Two to four moles of phosphate are fixed per mole of oxygen consumed. (c) The phosphate compounds formed are the main source of energy for the various forms

of work accomplished by the cell (d) The respiratory metabolism of isolated mitochondria, and presumably of tissues *in vivo*, is controlled in large part by the availability of inorganic phosphate and phosphate acceptors. Lardy and others have observed that mitochondrial preparations, when incubated with an oxidizable substrate and necessary cofactors, a phosphate buffer and phosphate acceptors and thyroxine bind more phosphate than such preparations not exposed to thyroxine. How-

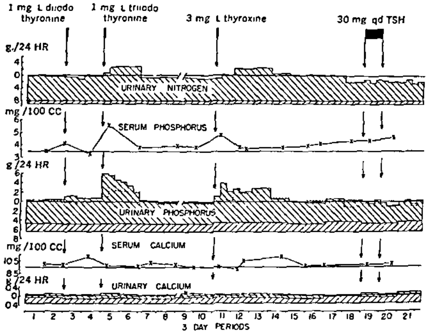



FIG 3a The effect of L-3 5 3′-triiodothyronine and of L-thyroxine on urinary excretion of nitrogen, phosphorus, and calcium in an adult myxedematous female

ever, since the oxygen consumption increases more rapidly than the phosphate fixed, he suggests that gains in oxygen consumption through the influence of thyroxine are at the expense of cellular efficiency.

There are certain disadvantages of this theory which merit comment. For example, these same changes can be produced by dinitrophenol, D-thyroxine (15), and halides such as iodide or bromide (17) It has been suggested, more recently, that this *in vitro* action of thyroxine is concerned with control of some structural property rather than the direct interaction of the hormone with the enzymes of oxidative phosphorylation (28). Finally the concentration of hormone required to produce

such *in vitro* effects is many times the level of hormones seen in the serum in severe hyperthyroidism.

On the other hand, Dr. Kathleen Roberts (24) and associates have demonstrated that the toxicity of 3.5:3′-triiodothyronine is demonstrable in dogs only after administering a phosphate load. In these studies it has

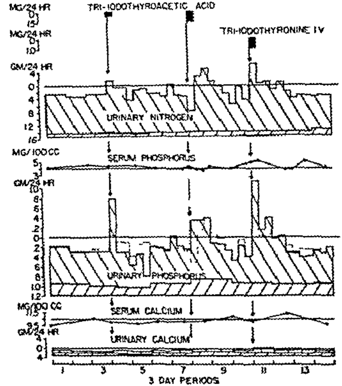



FIG. 3b The effect of triiodothyroacetic acid and of triiodothyronine on the urinary excretion of nitrogen, phosphorus, and calcium in an adult myxedematous male. The dotted lines in Figs. 3a and 3b represent the expected phosphorus excretion calculated from the urinary nitrogen and calcium excretion.

been demonstrated that the intravenous administration of triiodothyronine in doses up to 1 mg had no significant effect on the oxygen consumption of healthy dogs. However, if the animals are given an infusion of sodium phosphate prior to and during the administration of triiodothyronine, the animal has a marked and rapid increase in oxygen consumption and usually dies in a state which resembles thyroid storm.

These observations are not unlike those of Klemperer who has reported that the addition of triiodothyronine to mitochondrial preparations in appropriate media increased the incorporation of $P^{32}$ into the ATP system and its rate of turnover. This latter observation might also explain in part the marked and rapid urinary loss of phosphorus seen in myxematous patients treated with the various thyroid hormones that we have studied.

During this meeting we have heard repeated references to the retention of phosphorus which is observed in patients with schizophrenia. Dr. Hoagland has shown me some very interesting studies of the phosphate balance in such patients. He has found that the retention of phosphorus is reversed by stress or by the administration of ACTH. I hope that Dr. Hoagland will present these studies during the discussion In the light of our observation that triiodothyroacetic acid, a probable brain metabolite of thyroxine, when administered to a myxedematous patient in a dose that did not cause a rise in the rate of oxygen consumption caused a marked urinary loss of phosphorus, the desirability of evaluating the effects of thyroxine and all of its metabolites in schizophrenia becomes a challenging beckoning frontier.

Dr. Richter has described a pharmacologic effect of thyroxine and other preparations of thyroid hormone in rats with an habituation to alcohol. He has found that the administration of thyroxine or thyroid to these rats was followed by their choosing water in preference to alcohol. He has also observed that ablation of the thyroid increased the rats' appetite for alcohol. These observations complement those of Zarrow and Rosenberg (29) who have reported that they could increase the rats' appetite for alcohol by administering thiouracil. In the light of these observations it is of interest that the reported electroencephalographic pattern in myxedema with its diminished voltage and slow frequency is similar to that observed in many female and some male alcoholics in that there is an absence of alpha waves.

We have had occasion to observe a prompt sobering effect of triiodothyronine in a small group of acute alcoholics In collaboration with Dr. Diethelm and Dr Flack of the Payne Whitney Clinic, we have also observed the effects of triiodothyronine in four patients suffering with chronic alcoholism. Three of these patients experienced a decreased appetite for alcohol during treatment with triiodothyronine. Dr Flack will describe some of the psychiatric and metabolic changes observed in these patients.

## SUMMARY

1. We have reviewed the psychiatric problems observed in myxedematous patients and have presented preliminary observations made in induced athyrotic myxedema and in induced pituitary myxedema.

2. We have discussed the reported evidence for an hypothyroidism in certain psychotic patients

3 We have discussed the metabolic effects of certain thyroid hormones in myxedema and have attempted to correlate some of these changes with the phosphate retention which has been observed in schizophrenia.

4. We have proposed that the metabolic effects of the various known thyroid hormones and their metabolites be compared in patients with various psychiatric disorders.

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CHAIRMAN I C WINTER You are suggesting that this treatmnt increased the rate of metabolism of alcohol?

H J. KOCH, JR: I am sure it did, but I don't think that is the answer to the very acute sobering effect

R W. RAWSON. The effects in acute alcoholism that Dr Koch has discussed happened more rapidly than any change in the rate of oxygen consumption in humans we have been able to demonstrate It is possible, however, that such a dose of triiodothyronine might have produced a rapid change in water, salt, or even phosphate metabolism This will require further study

G PINCUS· What was the dose of the triiodothyronine used?

H J. KOCH, JR: In the first instance 200 μg and in the other instance 400 μg — the largest dose Dr. Rawson used in acute alcoholics which he observed

CHAIRMAN I C. WINTER· Does triiodothyronine (T3) do anything to ADH release?

H J. KOCH, JR. It probably does

R W. RAWSON. In answer to Dr Winter's question as to whether or not T3 influences the ADH So far as I know the effects of T3 on the ADH have not been studied The effects of thyroidectomy and of administered thyroid hormone have been investigated The studies in animals and humans are somewhat at variance I would say that at present we don't know.

R A CLEGHORN There is some evidence that ACTH will also help to sober up people and prevent hangover

A S MARRAZZI Is there any knowledge on what dinitrophenol does?

R W RAWSON In the *in vitro* experiments, dinitrophenol, a compound which does not alter the clinical picture of myxedema, exerts an influence on phosphorylation like that which results from the addition of thyroxine or triiodothyronine It would be most desirable to study the effect of dinitrophenol on the phosphate balance in myxedematous patients We have not studied its sobering effect

D W WOOLLEY At the International Biochemical Congress, Dr Pitt-Rivers pointed out last summer that many people know that the mitochondrial effect, uncoupling of phosphorylation by thyroxine, is difficult to demonstrate *in vitro* On the contrary, however, the propionic acid derived by removing the amino group from thyroxine lends itself to the ready demonstration of the phenomenon

R W RAWSON Recently it has been suggested that this effect is due to primary anatomical changes rather than to changes in function

D W WOOLLEY The point I wanted to bring out was that if one uses the propionic acid there is no trouble at all, and the effect can be demonstrated with no time lag This is usually not the case if one uses thyroxine.

R. W RAWSON Unfortunately other people have been unable to repeat Dr. Pitt-Rivers observations on the *in vitro* effects of triiodothyroacetic acid.

F. ELMADJIAN In your slide of the metabolic data of some of the compounds I don't think I saw anything about urine volume

R. W RAWSON I am sorry to say that we have not included on these charts the water loss The usual observed effect of a thyroid hormone in myxedema is a prompt diuresis We have observed this effect following the administration of all thyroid hormones that we have studied

F ELMADJIAN Would you care to restate some of the interpretation in regard

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## Discussion

H Hoagland· Curt Richter made a remark at the last Macy Conference on Neuropharmacology that triiodothyronine apparently was helpful in cases of hangovers I wonder if somebody has a comment about that

H J Koch, Jr I can make a brief comment concerning the effect of triiodothyronine on acute alcoholic intoxication In some preliminary observations of acutely intoxicated young men who were not chronic alcoholics, it was noted that triiodothyronine produced astonishingly rapid sobering effects The first observation was made on a 23-year-old male who had drunk eighteen martinis in the course of about eight hours He was comatose and showed lack of sphincter control and absence of pain reflex. A 100-µg tablet of triiodothyronine was held against the oral mucous membranes until it dissolved This took approximately five minutes and during this period the patient was noted to open his eyes and shake his head Within another ten minutes he was sitting up and was able to swallow another 100-µg. tablet of triiodothyronine Within half an hour after the first dose he was essentially sober He slept approximately eight hours and awoke with no hangover.

A second similar case was also observed to react in a like manner to triiodothyronine

Chairman I C Winter Were these subjects "normal" or people who were used to it, that is, chronic alcoholics?

H. J Koch, Jr The two cases that I just mentioned were both quite young and were not habituated to alcohol The patients observed became sober very rapidly and then fell into what appeared to be a normal sleep entirely unlike the restlessness usually observed in the acutely intoxicated individual

B B. Brodie The hangover did not have much alcohol left

H. J Koch, Jr. I agree with you Most people during hangover will show agitation, nervousness They did not show this Chronic alcoholics go into an acute episode.

add one iodine in the 3-position, it is still inactive, but if you transfer this iodine atom from the 5'-position over to the 5-position, it is the most active compound known (3,3',5-triiodothyronine). If you put one more iodine atom in 5' it is thyroxine and it is given the value of one. It is the position in which the iodines are placed on the benzene ring which is very important, it is not only that you have to use any iodinated compound

R. W. RAWSON: Yet if the iodine is completely replaced by four bromine atoms on the thyronine molecule, there is hormonal activity but one-tenth that of thyroxine; tetrachlorthyronine has about 1/200 the activity of thyroxine.

B. B. BRODIE. Has anybody tested the corresponding fluorinated compounds?

R. W. RAWSON. It has been tried and found noneffective in the doses available.

H. HOAGLAND: Dr Flach has had some experience with the administration of thyroid in relation to clinical psychopathologic and metabolic effects especially in alcoholics I would like to ask him to tell us about his investigations.

F. F. FLACH. The clinical and metabolic evaluations of the effectiveness of the thyroid hormone on the craving for alcohol, seen in certain alcoholic patients, is only part of a broader program of psychopathologic and physiologic investigations at the Payne Whitney Psychiatric Clinic. At the present time we are attempting to study correlations between nitrogen, creatine, and electrolyte balances and specific psychopathologic states This work takes cognizance of established diagnostic categories, but crosses diagnostic lines, so to speak, so that, for example, depression of mood, resentment, or thinking disorders are investigated as such wherever they may occur. Moreover, when possible, as in recurrent periodic illnesses or in the evaluations of metabolic alterations with therapy, the patient is used as his own control

The research environment is carefully controlled, but without interfering with the environmental therapeutic measures used to aid in the patients' recoveries Because each patient is also undergoing psychotherapy with the individual staff psychiatrist, it is possible to know the motivations and psychodynamic factors related to the patient's illness or influencing his emotional state during the course of duty.

The importance of this last factor cannot be emphasized too much As I discuss briefly the four patients who have received triiodothyronine for alcoholic problems, the role played by psychodynamic factors will become evident.

The first patient, a 23-year-old woman, received 100 μg of triiodothyronine per day, and described a subjective loss of craving for alcohol. She was, however, a somewhat unreliable reporter, and moreover was very poorly motivated to terminate her drinking at the time of discharge It was not surprising, therefore, that, whatever role the medication may have played, on leaving the hospital she promptly returned to drinking in spite of a maintenance dose of 50 μg. of triiodothyronine, subsequently raised to 100 μg without much benefit

The second patient, a woman of 40, who presented a picture of recurrent periods of heavy drinking in the setting of loneliness, tension, and concealed hostility, was given 100 μg per day and during this period of time seemed to improve in her social adjustment, with less evidence of withdrawal, tension, and irritability. The possibility of a rebound phenomenon is present, since there is a time correlation between the termination of the medication and an exacerbation of hostility, sexual preoccupations, and inappropriate mood elevation, necessitating temporary transfer

to the metabolic events that occur as the result of diuresis being primary or secondary to what is going on?

R. W. RAWSON: I am not certain that I can answer your question Several years ago Dr. Ranson and associates reported that the diabetes insipidus of cats having suprachiasmal lesions was decreased by removal of the thyroid but enhanced by the *replacement of thyroid hormone*. Doctors Pearson and Lipsett and MacLean of our institution have been unable to demonstrate any relationship between thyroid function and the diabetes insipidus which follows removal of the pituitary gland of humans with various types of cancer.

S. UDENFRIEND: The action of triiodothyroacetic acid on metamorphosis seems to be a most astounding thing Is there specificity in this respect? How about indole acetic acid and compounds of that sort? Are there many aromatic acids active?

R. W. RAWSON: I don't know. To me it is most interesting that some of these thyroid hormones and their derivatives should have such varied effects on different parameters of thyroid hormone actions. It appears that those compounds manifesting changes in the side chain have less effect on the rate of oxygen consumption and on the development of goiter but are more effective on the metamorphosis of the tadpole.

S. UDENFRIEND: That is what I meant to ask, whether it was specific?

R. W. RAWSON: There is not complete agreement as to the specificity of the assay as performed on tadpoles. I think we can say this method of assay simply measures one function of the thyroid hormone The recent studies to which I referred would indicate that the effects on metamorphosis of tadpoles does not reflect the capacity of a compound to alter the oxygen consumption Further study is needed before we can correlate these observations with other metabolic effects of these compounds.

D. W. WOOLLEY: We had occasion to test *N*-acetyl diiodotyrosine We could not find evidence of any activity on the tadpole Have you had experience with that?

R. W. RAWSON. We have not had the opportunity to do that

S. UDENFRIEND: There is one more question I want to ask Does iodide itself have any effect? You said you were getting effects in patients with triac when you gave large amounts. Would you have got any effects with comparable amounts of iodide?

R. W. RAWSON: No. It has been reported that the prolonged administration of iodides resulted in myxedema.

A. HOFFER. I understand that the indoles prevented metamorphosis of the tadpole. They are antagonistic to thyroid hormone

R. W. RAWSON. Adrenochrome does prevent it.

J. R TATA: I would like to make a comment in reference to what Dr. Udenfriend asked and that is that it is not only iodine that is important for these activities It is the position in which the iodine atom is present. If you ignore the side chain and work with the skeleton, and put the two iodines in the 3,5 positions, by any of the tests done so far no activity will result If, on the other hand, you put iodine in the 3'-position, the compound is very active. It is in fact as active as thyronine, if you measure it by the goiter-prevention test. If you

degree of retention With one notable exception, sodium was slightly lower, in the 23-year-old man with high nitrogen retention and periodic states of rage, alcohol consumption, depression, and thinking disorder, with concomitant physical symptoms of marked thirst and polyuria of considerable volume, a very high level of sodium retention was completely unaffected by triiodothyronine. Slight to moderate excretions of potassium were noted during treatment Of special interest were the excessive amounts of creatine in the urine of three of the four patients studied during the control periods

Since the preparation of this report, another patient has been studied for her reaction to triiodothyronine and triiodothyropropionic acid, product of thyroid metabolism. This patient was in a long-standing psychopathologic state, consisting of marked affective flatness, depersonalization, and depression of mood, in the setting of a schizophrenic reaction. On the administration of 200 μg of triiodothyronine there was an unquestionable elevation of mood, with diminished flatness of affect, decreased social withdrawal, and increased aggressiveness in daily activities These changes were even more marked when, following termination of triiodothyronine, triiodopropionic acid was administered in a low daily dosage of 0 2 mg Within 3 weeks, cheerfulness, affective responsiveness, and the disappearance of depersonalization were apparent BMR rose from an initially low level, cholesterol fell from a high level Metabolic studies demonstrated a definite loss of nitrogen and all electrolytes, of special interest was the fact that the loss of phosphorus seemed to exceed that which could be accounted for by the loss of nitrogen and calcium Creatinuria, initially high during control studies, decreased during triiodothyropropionic acid therapy in association with the most marked degree of clinical improvement Moreover, during the 2 weeks following termination of therapy, a gradual recurrence of mood depression and depersonalization was noted, without the same degree of affective blunting

No definite correlation between the administration of triiodothyronine, its metabolic effect and psychopathologic alterations can be made on the basis of these present studies However, the clinical and metabolic alterations observed during this preliminary investigation offer a number of avenues for further study, which we intend to pursue The principle of variability of response to a given hormonal substance based upon the pretreatment homeostatic level of metabolism appears to be important This concept seems to me to be of increasing significance in the search for inherent biochemical and physiological components of various psychopathologic states

R W Rawson. I asked Dr Hoagland yesterday if he would discuss here his findings concerning phosphorus metabolism in schizophrenics in relation to the adrenal cortex and the action of LSD.

H Hoagland *We have been concerned with a puzzling phenomenon in* relation to urinary inorganic phosphate excretion of chronic schizophrenics as compared to normal people What we have reported (Hoagland, H, Rinkel, M, and Hade, R W, *Arch Neurol Psychiat* 73, 100, 1955) in studies of a rather large number of schizophrenic patients and normal controls, matched for age, has been that the *schizophrenic at rest puts out about half of the inorganic phosphate* that one finds in the normal at rest and statistically these are very significant differences Now when one gives 25 mg of ACTH to schizophrenics and compares

to a more restricted environment. No evidence of drinking during or after drug administration was obtained She never described true cravings.

The third patient, a 23-year-old-man, had periods lasting several weeks during which time he would first become anxious and overactive in a well-organized way with some mood elevation, terminating in heavy drinking associated with rage outbursts, followed by retardation of speech and thought and depression of mood. After this he would undergo long periods of relative remission from these symptoms A disorder of thinking was present during the period of resentment and depression. On 100 μg of triiodothyronine he became less retarded, resentment and depression diminished, and his thinking disorder was minimized. Whether this was spontaneous clearing or related to drug administration is not clear. However, he continued to become more active and described accurately the subjective experience of being "keyed up" While still on medication, in the setting of a spite reaction against his therapist, he drank alcohol during a visit out of the clinic. He described a "lack of previously known pleasure" experienced with this episode of drinking. Corresponding in time to the termination of medication he too demonstrated what may be a rebound reaction, retardation of speech and thought and depression of mood returned, with strong resentment and thinking disorder lasting several weeks, and followed by gradual recovery in the setting of continued psychotherapy. Triiodothyronine was not reinstituted and drinking occurred following discharge, he left the clinic before therapy had reached any optimal point.

The fourth patient in this study, a 20-year-old man with a 6-year history of very heavy alcohol intake, was given 100 μg. of medication, which shortly thereafter had to be reduced to 50 μg on account of "jitters" presumably related to the dosage Initially he seemed to become more tense, irritable, and resentful, and less able to control the thought disorganization which was an intrinsic part of his psychopathology His electroencephalogram showed increasing disorganization of a nonspecific type Again the possibility of rebound phenomenon is present, since within 36 hours after termination of the medication, he became intoxicated during his first visit out of the hospital and paranoid suspiciousness became prominent.

Several clinical points are evident. The forces which drive each of these patients to alcohol differ, the pattern of drinking, the effect of alcohol, the dynamic motivation for controlling the habit, all vary extensively. Craving, as such, is rarely present, and in the one patient who described its disappearance while on medication, drinking continued anyway for highly dynamic reasons such as self-destruction or spite. Future investigation in this area would seem to demand more freedom to be exposed to alcohol, more specific selections of case material, and study of the effects of various dosages of triiodothyronine

The metabolic alterations are very interesting Summarizing them briefly, where nitrogen retention was initially high, marked depletion occurred during the administration of triiodothyronine, where it was initially low, depletion was slight All patients showed a rise in BMR and a fall in cholesterol during treatment With regard to electrolytes, where calcium was being retained, it was excreted on the medication; where it was being lost, it was retained; this pattern followed the sex distribution, the women, one pre- and one post-menopausal, showing an excretion of calcium during treatment. Phosphorus showed slight changes, in the direction of excretion, the degree of change appearing to be relative to the initial

Our data are consistent with the view that schizophrenics may produce a metabolite that acts like LSD both with respect to phosphate conjugation and its release by steroids, and also in its tendency to produce psychological disturbances Such an endogenous substance could result from derangements of catechol amine or of indole metabolism as discussed in some of the preceding papers. We are interested to see if the phosphate metabolism may thus serve as a link between experimentally induced psychotic states and clinical psychoses

B. B Brodie: If the cumulative retention of phosphate is calculated, schizophrenic patients should be almost pure phosphate in about 25 years. Were the patients and normal subjects receiving identical diets? Regarding the effects of LSD on phosphate excretion, is it possible that this agent constricts afferent arterioles to the kidneys and thereby changes kidney function?

H. Hoagland The chronic schizophrenic's phosphate excretion is very labile At rest he markedly retains phosphate, but when stressed he excretes it in excess Thus in the course of 24 hours his net excretion may be normal

Kidney function tests were not made by us, but in schizophrenics a lot of studies by others indicate that kidney function is quite normal These patients and the normals that were compared, were without breakfast in the morning, but were not under true basal conditions

B B. Brodie· What about LSD effects on the kidney?

H Hoagland: With the LSD there is the possibility that it may be interfering with kidney function in some way, and this should be further investigated Dietary factors could not account for the differences in the acute LSD experiments The schizophrenics ate the normal hospital diet and the controls had pretty much the same general diet.

B B Brodie How much did they eat?

H Hoagland: This was not controlled The depression of phosphate excretion is only under conditions of rest and quiescence. If the patients are stressed or active they accelerate excretion so there is a great deal of variability of phosphate excretion in the patients, i e, poor homeostasis.

E Anderson: Have they a different diurnal rhythm for phosphate excretion that normal people have?

H Hoagland I do not know

E. Anderson. The diurnal rhythm is disturbed in schizophrenics.

O. Diethelm Did you follow any of them over a period of time?

F Elmadjian Some years ago I followed the diurnal variation of inorganic phosphates in schizophrenia. There was nothing unusual about the findings. The phosphate value is elevated during sleep In the first few hours in the morning the values are low and there is a slight rise in the afternoon period. They will change according to diet and physical activity, e g, whether the subject is sitting down or lying down makes a difference, as well as whether or not he has just eaten Hyperglycemia will cause a decrease in inorganic phosphates of both urine and blood

A. S. Marrazzi· Did you need a special diet with the animals?

H Hoagland: The animals were on the standard laboratory diet throughout They did not have a special diet.

S S. Kety: Dr Rawson mentioned some data of Dr Gordan on the effects

the response of several measurements of adrenocortical function to those of normals given that same dose, one of the things we observed was that the excretion of inorganic phosphate in the schizophrenic bounds up, increasing 100% or more in response to the ACTH injection, whereas the normals as a group show no significant change, but a tendency to decrease phosphate excretion. This is not only true when ACTH is given but it is also found when adrenal cortical extract is injected or when the subject is stressed and the adrenal cortex is stimulated endogenously. There is an abnormally low basal phosphate output in the schizophrenic with a marked increase in response to adrenocorticoids, whether from ACTH administration or direct injection of corticoids or by stress and this is not the case in the normal population. I think this is the most striking difference we have seen statistically between populations of schizophrenics and normals. This puzzled us for a long time and when I had an opportunity to do some work with Dr. Rinkel and Dr. Hyde on LSD effects in normals at the Boston Psychopathic Hospital, we made a collaborative study to see if there were parallels between aspects of adrenal cortical responsivity as measured in the urine of normals under the influence of LSD and schizophrenics without any drug. We investigated this by studying the effects of injected LSD on the output of 17-ketosteroids, electrolytes, including inorganic phosphates, and the effect of ACTH administered during the time of action of the LSD and in its absence in 22 experiments on 11 normal men In other words, LSD would be given and some hours later at the time of its psychotomimetic action we would give ACTH and observe the effect on phosphate excretion and some of the other responses What we saw here was that the phosphate excretion was cut down in those patients under the influence of LSD, by about 50% for a 50-µg. dose of LSD and then continued for some hours to be excreted at a low level. When we gave ACTH during the time of action of the LSD there was a marked increased output of phosphate, very much like what we had seen in the schizophrenics without drug. Dr. John Bergen, in our laboratory, has carried out some studies with guinea pigs along these lines. He has investigated the effect of injection of LSD in the guinea pig's phosphate excretion. He finds that LSD reduces this markedly and that, as in man, ACTH produces a marked enhancement of output during the time of action of LSD.

From these various observations we have tended to hypothesize that there may be enhanced conjugation of phosphates, perhaps in the form of hexose monophosphate since Meyer-Gross has presented evidence that this substance is increased as a result of the action of LSD Our hypothesis further is that steroids may release this conjugated phosphate so that on stress or ACTH administration it pours out in the urine. We are trying to investigate this further now by direct analysis of conjugated phosphorylated compounds in blood of guinea pigs, and also in normals under LSD and following ACTH, and in schizophrenic patients without LSD but following ACTH injections

Elmadjian has criticized this hypothesis on the grounds that LSD releases adrenaline as he has shown in man, and that the adrenaline then depresses the phosphate output so that we are simply measuring the effects of released adrenaline But this is not consistent with the fact that the guinea pigs show increasing depressions of phosphate excretion with increasing doses of LSD up to 50 µg, but with greater doses the depressing effect decreases and disappears with 150 µg. Autonomic effects continually increase the larger the dose.

Our data are consistent with the view that schizophrenics may produce a metabolite that acts like LSD both with respect to phosphate conjugation and its release by steroids, and also in its tendency to produce psychological disturbances Such an endogenous substance could result from derangements of catechol amine or of indole metabolism as discussed in some of the preceding papers. We are interested to see if the phosphate metabolism may thus serve as a link between experimentally induced psychotic states and clinical psychoses.

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H. HOAGLAND: The animals were on the standard laboratory diet throughout. They did not have a special diet

S S KETY: Dr. Rawson mentioned some data of Dr. Gordan on the effects

of some of these thyroid hormones in schizophrenia I wonder if there is time for that

CHAIRMAN I. C WINTER I think so. Dr Gordan has indicated reluctance to comment, but perhaps we can talk him into it.

G. S GORDAN: The reluctance is for the simple reason that the current cerebral blood flow techniques do not permit one to draw conclusions from anything less than statistically valid groups Our publications on cerebral blood flow and cerebral oxygen uptake in endocrinopathies have been restricted to series where we have very large groups. We have already reported high oxygen uptakes in hypopituitary and hypogonadal patients (Gordan, G. S, and Adams, J E *In* "Hormones and the Aging Process" (E T. Engle, and G Pincus, eds.), p 299. Academic Press, New York, 1956) We think that the steroids probably constitute *a normal restraint upon the oxygen and glucose utilization of the brain and* this explains the normal drop in these rates at puberty In our series, no further change occurs with aging, at least up to age 91

It is curious that hypopituitary patients who have low total body oxygen uptake—low BMR—have a very high cerebral metabolic rate for oxygen. There are four reports on cerebral metabolism in hypo- and hyperthyroidism, and four sets of conflicting results, of which the most recent is that of Sensenbach, in *J. Clin Invest.* **33**, 1434, 1954 If his data, which are very extensive, are to be accepted, neither hyperthyroidism nor hypothyroidism significantly affects oxygen or glucose utilization

We ran into one controversy, in that Dr Kety has reported normal oxygen uptake in patients with chronic schizophrenia, whereas we find that patients who have had the disease for a long time have low cerebral oxygen uptakes I don't know the reason for this discrepancy because I use Dr. Kety's original unmodified technique, and in last year's conference I inveighed against the modifications of this technique, which I think have taken all the internal checks out of it. As for the effect of the triiodothyroacetic acid in cerebral metabolism, Dr. Rawson reported our observations correctly The only reservation is that we have had very few subjects so far.

# Author Index

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